	Enzyme	Cleavage of 75 Recognition*	Nch i		
	AfeI		0	0	Flanned Tocacton of Site
	AflII	-	ŏ	ō	HC FR3
5	AgeI	Accggt	Ö	0	10 110
•	AscI	GGcgcgcc	ŏ	0	After LC
		Agatct	0	0	After LC
		Cgtacg		0	
		ATcgat	0 0	0	
10		Gegege	0	0	
		TTcgaa			
	DSCDI	CACNNNgtg	0 0	0	
	EagI				
		Cggccg GGCCGGcc	0	0	
15			0 0	0	
		TGCgca GTTaac		0	
	HpaI MfeI		0	0	
		Caattg	0	0	HC FR1
	MluI	Acgcgt	0	0	
20	NcoI	Ccatgg	0		Heavy chain signal
20	NheI	Gctagc	0	0	HC/anchor linker
	NotI	GCggccgc	0	0	In linker after HC
	NruI	TCGcga	0	0	
.e. en.	PacI	TTAATtaa	0	0	
[]_	PmeI	GTTTaaac	0	0	·
₹25		CACgtg	0	0	
ţ0		CGATcg	0	0	
Lil .	SacII	CCGCgg	0	0	
1.1	SalI	Gtcgac	0	0	
30	SfiI	GGCCNNNNnggcc	0	0	Heavy Chain signal
<u> </u>	SgfI	GCGATcgc	0	0	•
1.2	SnaBI	TACgta	0	0	
ţħ.	StuI	AGGcct	0	0	
3	XbaI	Tctaga	0	0	HC FR3
[<u>]</u> 25	AatII	GACGTc	1	1	
ຼູ <u>ສ</u> ຼັ 5	AclI	AAcgtt	1	1	
ļ.a.	AseI	ATtaat	1	1	
F	BsmI	GAATGCN	1	1	
141	BspEI	Tccgga	1	1	HC FR1
	BstXI	CCANNNNntgg	1		HC FR2
‡40	DrdI	GACNNNNnngtc	1	1	
	HindIII	Aagctt	1	1	
	PciI	Acatgt	1	1	
	SapI	gaagagc	1	1	
	Scal	AGTact	1	1	•
45	SexAI	Accwggt	1	1	·
	SpeI	Actagt	1	1	
	TliI	Ctcgag	1	1	
	XhoI	Ctcgag	1	1	
	BcgI	cgannnnnntgc	2	2	
	BlpI	GCtnagc	2	2	
50	DIPI		2	2	
50	BssSI	Ctcgtg	_		
50		Ctcgtg GCANNNNntgc	2	2	
50	BssSI		2	2	
	BssSI BstAPI	GCANNNNntgc	2	2	
50 55	BssSI BstAPI EspI	GCANNNNntgc GCtnagc	2 2 2	2	
	BssSI BstAPI EspI KasI	GCANNNNntgc GCtnagc Ggcgcc	2 2 2 2	2 2 2	

	ApaLI	Gtgcac	3	3	LC signal seq
	Nael	GCCggc	3	3	
	NgoMI		3	3	
	PvuII		3 3	3	
5	RsrII	CGgwccg	3	3	
	BsrBI	GAGcgg	4	4	
	BsrDI	GCAATGNNn	4	4	
	BstZ17I	GTAtac	4	4	
	EcoRI	Gaattc	4	4	
10	SphI	GCATGC	4	4	
	SspI	AATatt	4	4	
	AccI	GTmkac	5	5	
	BclI	Tgatca	5	5	
	BsmBI	Nnnnngagacg	5	5	
15	BsrGI	Tgtaca	5	5	
	DraI	TTTaaa	6	6	
	NdeI	CAtatg	6	6	HC FR4
	SwaI	ATTTaaat	6	6	
	BamHI	Ggatcc	7	7	
20	SacI	GAGCTc	7	7	
	BciVI	GTATCCNNNNNN	8	8	
	BsaBI	GATNNnnatc	8	8	
	NsiI	ATGCAt	8	8	
73	Bsp120I	Gggccc	9	9	CH1
[] 25	ApaI	GGGCCc	9	9	CH1
Ü	PspOOMI	Gggccc	9	9	
tu Lu	BspHI	Tcatga	9	11	
	EcoRV	GATatc	9	9	
. if	AhdI	GACNNNnngtc	11	11	
30	BbsI	GAAGAC	11	14	
IJ	PsiI	TTAtaa	12	12	
(A.	BsaI	GGTCTCNnnnn	13	15	
	Xma I	Cccggg	13	14	
35	AvaI	Cycgrg	14	16	
	BglI	GCCNNNNnggc	14	17	
= = = .	AlwNI		16	16	
} =	BspMI	ACCTGC	17	19	
4.1	XcmI	CCANNNNnnnntgg	17	26	
O	BstEII	Ggtnacc	19	22	HC FR4
40	Sse8387I	CCTGCAgg	20	20	
	AvrII	Cctagg	22	22	
	HincII	GTYrac	22	22	
	BsgI	GTGCAG	27	29	
4-	MscI	TGGcca	30	34	
45	BseRI	NNnnnnnnnctcctc	32	35	
	Bsu36I	CCtnagg	35	37	
	PstI	CTGCAg	35	40	
	EciI	nnnnnnnntccgcc	38	40	
ΕΛ.	PpuMI	RGgwccy	41	50	
50	StyI	Ccwwgg	44	73	
	Eco0109I	RGgnccy	46	70	
	Acc65I	Ggtacc	50	51	
	KpnI	GGTACC	50	51	
55	BpmI	ctccag	53	82	
J	AvaII	Ggwcc	11	124	

 $[\]boldsymbol{\star}$ cleavage occurs in the top strand after the last upper-case base. For REs

that cut palindromic sequences, the lower strand is cut at the symmetrical site.

Table 2: Cleavage of 79 human heavy chains

5	Table 2: 0	Cleavage of 79 hum	nan hea	avy	chains
•	Enzyme	Recognition	Nch	Ns	Planned location of site
	AfeI	AGCgct	0	0	
	AflII	Cttaag	0	0	HC FR3
	AscI	GGcgcgcc	0	0	After LC
	BsiWI	Cgtacg	0	0	
	BspDI	ATcgat	Ö	0	
	BssHII	Gcgcgc	Ö	Ö	
	FseI	GGCCGGcc	Ö	Ö	
	HpaI	GTTaac	ŏ	0	·
	NheI	Gctagc	ŏ	Ö	HC Linker
	NotI	GCggccgc	Ö	0	In linker, HC/anchor
	NruI	TCGcga	0	0	•
	NsiI	ATGCAt	Ö	Ö	
	PacI	TTAATtaa	Ö	ō	
	PciI	Acatgt	Ö	ŏ	
	PmeI	GTTTaaac	Ö	Ö	
	PvuI	CGATcg	ŏ	0	
	RsrII	CGgwccg	ő	Ö	
	SapI	gaagagc	Ö	0	
	SfiI	GGCCNNNNnggcc	ŏ	ŏ	HC signal seq
	SgfI	GCGATcgc	Ŏ	0	Jighar Beq
	SwaI	ATTTaaat	Ö	0	
	AclI	AAcgtt	1	1	
	AgeI	Accggt	1	1	
	AseI	ATtaat	1	î	
	AvrII	Cctagg	1	1	
	BsmI	GAATGCN	ī	ī	
	BsrBI	GAGcgg	ī	1	
	BsrDI	GCAATGNNn	ī	1	
	DraI	TTTaaa	ī	ī	
	FspI	TGCgca	ī	1	
	HindIII	Aagctt	ī	1	
	MfeI	Caattg	ī		HC FR1
	NaeI	GCCggc	1	1	nc eri
	NgoMI	Gccggc	1	1	
	SpeI	Actagt	1	1	
	Acc65I	Ggtacc	2	2	
	BstBI	TTcgaa	2	2	
	KpnI	GGTACc	2	2	
	MluI	Acgcgt	2		
	Ncol	Ccatgg	2	2 2	7- VO
	NdeI				In HC signal seq
		CAtatg	2	2	HC FR4
	PmlI	CACgtg	2	2	
	XcmI	CCANNNNnnnntgg	2	2	
	BcgI	cgannnnnntgc	3	3	
	BclI	Tgatca	3	3	
	BglI	GCCNNNNnggc	3	3	
	BsaBI	GATNNnnatc	3	2 3 3 3 3 3	
	BsrGI	Tgtaca	3	3	
	SnaBI	TACgta	3	3	
	Sse8387I	CCTGCAgg	3	3	

	ApaLI	Gtgcac	4	4	LC Signal/FR1
	BspHI	Tcatga	4	4	
	BssSI	Ctcgtg	4	4	
	PsiI	TTAtaa	4	5	
5	SphI	GCATGC	4	4	
	AhdI	GACNNNnngtc	5	5	
	BspEI	Teegga	5	5	
	MscI	TGGcca	5	5	
	SacI	GAGCTC	5	5	
10	Scal	AGTact	5	5	
	SexAI	Accwggt	5	6	
	SspI	AATatt	5	5	
	TliI	Ctcgag	5	5	
	XhoI	Ctcgag	5	5	
15	BbsI	GAAGAC	7	8	
	BstAPI	GCANNNNntgc	7	8	
	BstZ17I	GTAtac	7	7	
	EcoRV	GATatc	7	7	
	EcoRI	Gaattc	8	8	
20	BlpI	GCtnagc	9	9	
	Bsu36I	CCtnagg	9	9	
	DraIII	CACNNNgtg	9	9	
	EspI	GCtnagc	9	9	
f 1	StuI	AGGcct	9	13	
25	XbaI	Tctaga	9	9	
Ö	Bsp120I	Gggccc	10	11	CH1
t di	ApaI	GGGCCc	10	11	
Min din	PspOOMI	Gggccc	10	11	
74	BciVI	GTATCCNNNNNN	11	11	
30	SalI	Gtcgac	11.	12	
13	DrdI	GACNNNnngtc	12	12	
M	KasI	Ggcgcc	12	12	
5	Xma I	Cccggg	12	14	
= =.	BglII	Agatct	14	14	
35	HincII	GTYrac	16	18	
1 :	BamHI	Ggatcc	17	17	
ļ.	PflMI	CCANNNNntgg	17	18	
4	BsmBI	Nnnnnngagacg	18	21	
40	BstXI	CCANNNNntgg	18	19	HC FR2
$\mu \nu$	XmnI	GAANNnnttc	18	18	
	SacII	CCGCgg	19	19	
	PstI	CTGCAg	20	24	
	PvuII	CAGctg	20	22	
45	AvaI	Cycgrg	21	24	
45	EagI	Cggccg	21	22	
	AatII	GACGTC	22	22	
	BspMI	ACCTGC	27	33	
	AccI	GTmkac	30	43	
EΛ	StyI	Ccwwgg	36	49	
50	AlwNI	CAGNNNctg	38	44	
	BsaI	GGTCTCNnnnn	38	44	
	PpuMI	RGgwccy	43	46	
	BsgI	GTGCAG	44	54	
55	BseRI	NNnnnnnnnctcctc	48	60	
<i></i>	EciI	nnnnnnnntccgcc	52	57	
	BstEII	Ggtnacc	54	61	HC Fr4, 47/79 have one
	Eco01091	RGgnccy	54	86	

The first state of the state of

```
Table 5(amended): Use of FokI as "Universal Restriction Enzyme"
FokI - for dsDNA, | represents sites of cleavage
                           sites of cleavage
     5'-cac<u>GGATG</u>tg--nnnnnnn|nnnnnn-3'(SEQ ID NO:15)
     3'-gtgCCTACac--nnnnnnnnnnnnnnn-5'(SEQ ID NO:16)
           RECOG
           NITion of FokI
Case I
              5'-...gtg|tatt-actgtgc..Substrate....-3' (SEQ ID NO:17)
                 3'-cac-ataa|tgacacg-
                                     gtGTAGGcac\
                                  5'- caCATCCgtg/(SEQ ID NO:18)
Case II
              5'-...gtgtatt|agac-tgc..Substrate....-3'(SEQ ID NO:19)
                  r-cacataa-tctg|acg-5'
        /gtgCCTACac
        \cacGGATGtg-3'(SEQ ID NO:20)
Case III (Case I rotated 180 degrees)
        /gtgCCTACac-5'
        \cacGGATG<u>tq</u>
                    gtgtctt|acag-tcc-3' Adapter (SEQ ID NO:21)
              3'-...cacagaa-tgtc|agg..substrate....-5'(SEQ ID NO:22)
Case IV (Case II rotated 180 degrees)
```

```
3'- gtGTAGGcac\ (SEQ ID NO:23)
                                    _caCATCCgtg/
                 5'-gag|tctc-actgage
    Substrate 3'-...ctc-agag|tgactcg...-5'(SEQ ID NO:24)
Improved FokI adapters
FokI - for dsDNA, | represents sites of cleavage
Case I
Stem 11, loop 5, stem 11, recognition 17
           5'-...catgtg|tatt-actgtgc..Substrate....-3'
              3'-gtacac-<u>ataa|tgacacg</u>-
                                      gtGTAGGcacG T
                                  5'- caCATCCgtgc C
Case II
Stem 10, loop 5, stem 10, recognition 18
              5'-...gtgtatt|agac-tgctgcc..Substrate....-3'
              _<u>cacataa</u>-tctg|acgacgg-5'
      T gtgCCTACac
        cacGGATGtg-3'
Case III (Case I rotated 180 degrees)
Stem 11, loop 5, stem 11, recognition 20
     T TgtgCCTACac-5'
     G AcacGGATGtq
                   gtqtctt|acag-tccattctg-3' Adapter
              3'-...cacagaa-tgtc|aggtaagac..substrate....-5'
Case IV (Case II rotated 180 degrees)
Stem 11, loop 4, stem 11, recognition 17
                                                \Gamma^{T}
                                  3'- gtGTAGGcacc T
                                    <u>ca</u>CATCCgtgg T
              5'-atcgag|<u>tctc-actgagc</u>
 Substrate 3'-...tagctc-agag|tgactcg...-5'
```

BseRI

```
| sites of cleavage

5'-cacGAGGAGnnnnnnnnn|nnnn-3'
3'-gtgctcctcnnnnnnnn|nnnnn-5'
RECOG
NITion of BseRI

Stem 11, loop 5, stem 11, recognition 19

3'-....gaacat|cg-ttaagccagta....5'

[T-T] cttgta-gc|aattcggtcat-3'
C GCTGAGGAGTC-J
T cgactcctcag-5' An adapter for BseRI to cleave the substrate above.
```

Table 8: Matches to URE FR3 adapters in 79 human Ho	Table	8:	Matches	to	URE	FR3	adapters	in	79	human	HC
---	-------	----	---------	----	-----	-----	----------	----	----	-------	----

	A. List of	Heavy-chains	genes sampled		
	AF008566	af103343	HSA235676	HSU92452	HSZ93860
	AF035043	AF103367	HSA235675	HSU94412	HSZ93863
5	AF103026	AF103368	HSA235674	HSU94415	MCOMFRAA
	af103033	AF103369	HSA235673	HSU94416	MCOMFRVA
	AF103061	AF103370	HSA240559	HSU94417	S82745
	Af103072	af103371	HSCB201	HSU94418	S82764
	af103078	AF103372	HSIGGVHC	HSU96389	S83240
10	AF103099	AF158381	HSU44791	HSU96391	SABVH369
	AF103102	E05213	HSU44793	HSU96392	SADEIGVH
	AF103103	E05886	HSU82771	HSU96395	SAH2IGVH
	AF103174	E05887	HSU82949	HSZ93849	SDA3IGVH
17	AF103186	·HSA235661	HSU82950	HSZ93850	SIGVHTTD
	af103187	HSA235664	HSU82952	HSZ93851	SUK4IGVH
U	AF103195	HSA235660	HSU82961	HSZ93853	
`4 Lj	af103277	HSA235659	HSU86522	HSZ93855	
j.	af103286	HSA235678	HSU86523	HSZ93857	
	AF103309	HSA235677			

Table 8 B. Testing all distinct GLGs from bases 89.1 to 93.2 of the heavy variable domain

II.	Id	Nb	0	1	2	3	4		SEQ ID NO:
ij	1	38	15	11	10	0	2	Seq1 gtgtattactgtgc	25
}±	2	19	7	6	4	2	0	Seq2 gtAtattactgtgc	26
	3	1	0	0	1	0	0	Seq3 gtgtattactgtAA	27
25	4	7	1	5	1	0	0	Seq4 gtgtattactgtAc	28
	5	0	0	0	0	0	0	Seq5 Ttgtattactgtgc	29
	6	0	0	0	0	0	0	Seq6 TtgtatCactgtgc	30
	7	3	1	0	1	1	0	Seq7 ACAtattactgtgc	31
	8	2	0	2	0	0	0	Seq8 ACgtattactgtgc	32
30	9	9	2	2	4	1	0	Seq9 ATgtattactgtgc	33
	Group		26	26	21	4	2		
	Cumulative		26	52	73	77	79		

Table 8C Most important URE recognition segs in FR3 Heavy VHSzy1 GTGtattactgtgc (ON SHC103) (SEQ ID NO:25) 1 GTAtattactgtgc 2 VHSzy2 (ON SHC323) (SEQ ID NO:26) 3 VHSzy4 GTGtattactgtac (ON SHC349) (SEQ ID NO:28) 5 VHSzy9 (ON SHC5a) ATGtattactgtgc (SEQ ID NO:33) Table 8D, testing 79 human HC V genes with four probes Number of sequences..... 79 Number of bases..... 10 Number of mismatches Ιd 0 1 2 3 4 5 Best 2 1 O Seq1 gtgtattactgtgc (SEQ ID NO:25) 1 39 15 11 10 2 1 Seg2 gtAtattactgtgc (SEQ ID NO:26) 22 7 6 5 3 0 15 3 7 1 5 1 0 0 0 Seq4 gtgtattactgtAc (SEQ ID NO:28) 4 0 Seq9 ATqtattactqtqc (SEQ ID NO:33) 11 25. 26 20 5 2 Group T 25 51 71 76 78 Cumulative

One sequence has five mismatches with sequences 2, 4, and 9; it is scored as best for 2.

Id is the number of the adapter.

20 -----

"""" "**"""" "5**"""

Ü

14

Best is the number of sequence for which the identified adapter was the best available.

The rest of the table shows how well the sequences match the adapters. For example, there are 11 sequences that match VHSzy1(Id=1) with 2 mismatches and are worse for all other adapters. In this sample, 90% come within 2 bases of one of the four adapters.

```
Table 130: PCR primers for amplification of human Ab genes
                     5'-tgg aag agg cac gtt ctt ttc ttt-3'
      !(HuIgMFOREtop)5'-aaa gaa aag aac gtg cct ctt cca-3' = reverse complement
      (HuCkFOR)
                     5'-aca ctc tcc cct gtt gaa gct ctt-3'
      (HuCL2FOR)
                     5'-tga aca ttc tgt agg ggc cac tg-3'
      (HuCL7FOR)
                     5'-aga gca ttc tgc agg ggc cac tg-3'
O
ļż
      ! Kappa
 35
      (CKForeAsc) 5'-acc gcc tcc acc ggg cgc gcc tta tta aca ctc tcc cct gtt-
                     gaa gct ctt-3'
      (CL2ForeAsc)
                     5'-acc gcc tcc acc ggg cgc gcc tta tta tga aca ttc tgt-
                     agg ggc cac tg-3'
                     5'-acc gcc tcc acc ggg cgc gcc tta tta aga gca ttc tgc-
      (CL7ForeAsc)
 40
                     agg ggc cac tg-3'
```

! 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80

Table 195: Human GLG FR3 sequences

45

! VH1

agg gtc acc atg acc agg gac acg tcc atc agc aca gcc tac atg ! 81 82 82a 82b 82c 83 84 85 86 87 88 89 90 91 gag ctg agc agg ctg aga tct gac gac acg gcc gtg tat tac tgt gcg aga ga ! 1-02# 1 aga gtc acc att acc agg gac aca tcc gcg agc aca gcc tac atg gag ctg agc agc ctg aga tct gaa gac acg gct gtg tat tac tgt gcg aga ga ! 1-03# 2 aga gtc acc atg acc agg aac acc tcc ata agc aca gcc tac atg gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt gcg aga gg ! 1-08# 3 aga gtc acc atg acc aca gac aca tcc acg agc aca gcc tac atg gag ctg agg agc ctg aga tct gac gac acg gcc gtg tat tac tgt gcg aga ga ! 1-18# 4 aga gtc acc atg acc gag gac aca tct aca gac aca gcc tac atg gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt gca aca ga ! 1-24# 5 aga gtc acc att acc agg gac agg tct atg agc aca gcc tac atg gag ctg agc agc ctg aga tct gag gac aca gcc atg tat tac tgt gca aga ta ! 1-45# 6 aga gtc acc atg acc agg gac acg tcc acg agc aca gtc tac atg gag ctg agc ctg aga tct gag gac acg gcc gtg tat tac tgt

aga gtc acc att acc agg gac atg tcc aca agc aca gcc tac atg gag ctg agc agc ctg aga tcc gag gac acg gcc gtg tat tac tgt gcg gca ga ! 1-58# 8 aga gtc acg att acc gcg gac gaa tcc acg agc aca gcc tac atg 5 gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt gcg aga ga ! 1-69# 9 aga gtc acg att acc gcg gac aaa tcc acg agc aca gcc tac atg gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt gcg aga ga ! 1-e# 10 10 aga gtc acc ata acc gcg gac acg tct aca gac aca gcc tac atg gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt gca aca ga ! 1-f# 11 ! VH2 agg ctc acc atc acc aag gac acc tcc aaa aac cag gtg gtc ctt 15 aca atg acc aac atg gac cct gtg gac aca gcc aca tat tac tgt gca cac aga c! 2-05# 12 O agg ctc acc atc tcc aag gac acc tcc aaa agc cag gtg gtc ctt 13 acc atg acc aac atg gac cct gtg gac aca gcc aca tat tac tgt £9 gca cgg ata c! 2-26# 13 agg ctc acc atc tcc aag gac acc tcc aaa aac cag gtg gtc ctt aca atg acc aac atg gac cct gtg gac aca gcc acg tat tac tgt ĪĪ gca cgg ata c! 2-70# 14 3 ! VH3 [] [] []25 cga ttc acc atc tcc aga gac aac gcc aag aac tca ctg tat ctg caa atg aac agc ctg aga gcc gag gac acg gct gtg tat tac tgt gcg aga ga ! 3-07# 15 cga ttc acc atc tcc aga gac aac gcc aag aac tcc ctg tat ctg caa atg aac agt ctg aga gct gag gac acg gcc ttg tat tac tgt gca aaa gat a! 3-09#16 30 cga ttc acc atc tcc agg gac aac gcc aag aac tca ctg tat ctg caa atg aac agc ctg aga gcc gag gac acg gcc gtg tat tac tgt gcg aga ga ! 3-11# 17 cga ttc acc atc tcc aga gaa aat gcc aag aac tcc ttg tat ctt caa atg aac agc ctg aga gcc ggg gac acg gct gtg tat tac tgt 35 gca aga ga ! 3-13# 18 aga ttc acc atc tca aga gat gat tca aaa aac acg ctg tat ctg caa atg aac agc ctg aaa acc gag gac aca gcc gtg tat tac tgt acc aca ga ! 3-15# 19 cga ttc acc atc tcc aga gac aac gcc aag aac tcc ctg tat ctg

.

caa atg aac agt ctg aga gcc gag gac acg gcc ttg tat cac tgt gcg aga ga ! 3-20# 20 cga ttc acc atc tcc aga gac aac gcc aag aac tca ctg tat ctg caa atg aac agc ctg aga gcc gag qac acg gct gtg tat tac tgt 5 gcg aga ga ! 3-21# 21 cgg ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg caa atg aac agc ctg aga gcc gag gac acg gcc gta tat tac tqt gcg aaa ga ! 3-23# 22 cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg 10 caa atg aac agc ctg aga gct gag gac acg gct gtg tat tac tgt gcg aaa ga ! 3-30# 23 cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg caa atg aac agc ctg aga gct gag gac acg qct gtg tat tac tgt gcg aga ga ! 3303# 24 15 cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg caa atg aac agc ctg aga gct gag gac acg gct gtg tat tac tgt [] gcg aaa ga ! 3305# 25 Ę cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg [0 caa atg aac agc ctg aga gcc gag gac acg gct gtg tat tac tgt LJ gcg aga ga ! 3-33# 26 Lj cga ttc acc atc tcc aga gac aac agc aaa aac tcc ctg tat ctg T M caa atg aac agt ctg aga act gag gac acc gcc ttg tat tac tgt gca aaa gat a! 3-43#27 [] [] []25 cga ttc acc atc tcc aga gac aat gcc aag aac tca ctg tat ctg caa atg aac agc ctg aga gac gag gac acg gct gtg tat tac tgt gcg aga ga ! 3-48# 28 aga ttc acc atc tca aga gat ggt tcc aaa agc atc gcc tat ctg caa atg aac agc ctg aaa acc gag gac aca gcc gtg tat tac tgt act aga ga ! 3-49# 29 30 cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctt caa atg aac agc ctg aga gcc gag gac acg gcc gtg tat tac tgt gcg aga ga ! 3-53# 30 aga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctt caa atg ggc agc ctg aga gct gag gac atg gct gtg tat tac tgt 35 gcg aga ga ! 3-64# 31 aga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctt caa atg aac agc ctg aga gct gag gac acg gct gtg tat tac tgt gcg aga ga ! 3-66# 32 aga ttc acc atc tca aga gat gat tca aag aac tca ctg tat ctg

caa atg aac agc ctg aaa acc gag gac acg gcc gtg tat tac tgt gct aga ga ! 3-72# 33 agg ttc acc atc tcc aga gat gat tca aag aac acg gcg tat ctg caa atg aac agc ctg aaa acc gag gac acg gcc gtg tat tac tgt 5 act aga ca ! 3-73# 34 cga ttc acc atc tcc aga gac aac gcc aag aac acg ctg tat ctg caa atg aac agt ctg aga gcc gag gac acg gct gtg tat tac tgt gca aga ga ! 3-74# 35 aga ttc acc atc tcc aga gac aat tcc aag aac acg ctg cat ctt 10 caa atg aac agc ctg aga gct gag gac acg gct gtg tat tac tgt aag aaa ga ! 3-d# 36 ! VH4 cga gtc acc ata tca gta gac aag tcc aag aac cag ttc tcc ctg aag ctg agc tct gtg acc gcc gcg gac acg gcc gtg tat tac tgt 15 gcg aga ga ! 4-04# 37 cga gtc acc atg tca gta gac acg tcc aag aac cag ttc tcc ctg The second secon aag ctg agc tct gtg acc gcc gtg gac acg gcc gtg tat tac tgt gcg aga aa ! 4-28# 38 cga gtt acc ata tca gta gac acg tct aag aac cag ttc tcc ctg aag ctg agc tct gtg act gcc gcg gac acg gcc gtg tat tac tgt gcg aga ga ! 4301# 39 cga gtc acc ata tca gta gac agg tcc aag aac cag ttc tcc ctg aag ctg agc tct gtg acc gcc gcg gac acg gcc gtg tat tac tgt #.# 0..9 gcc aga ga ! 4302# 40 cga gtt acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg aag ctg agc tct gtg act gcc gca gac acg gcc gtg tat tac tgt 7 gcc aga ga ! 4304# 41 Ü ļè cga gtt acc ata tca gta gac acg tct aag aac cag ttc tcc ctg aag ctg agc tct gtg act gcc gcg gac acg gcc gtg tat tac tgt 30 gcg aga ga ! 4-31# 42 cga gtc acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg aag ctg agc tct gtg acc gcc gcg gac acg gct gtg tat tac tgt gcg aga ga ! 4-34# 43° cga gtc acc ata tcc gta gac acg tcc aag aac cag ttc tcc ctg 35 aag ctg agc tct gtg acc gcc gca gac acg gct gtg tat tac tgt gcg aga ca ! 4-39# 44 cga gtc acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg aag ctg agc tct gtg acc gct gcg gac acg gcc gtg tat tac tgt gcg aga ga ! 4-59# 45

cga gtc acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg aag ctg agc tct gtg acc gct gcg gac acg gcc gtg tat tac tgt gcg aga ga ! 4-61# 46 cga gtc acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg 5 aag ctg agc tct gtg acc gcc gca gac acg gcc gtg tat tac tgt gcg aga ga ! 4-b# 47 ! VH5 cag gtc acc atc tca gcc gac aag tcc atc agc acc gcc tac ctg cag tgg agc agc ctg aag gcc tcg gac acc gcc atg tat tac tgt 10 gcg aga ca ! 5-51# 48 cac gtc acc atc tca gct gac aag tcc atc agc act gcc tac ctg cag tgg agc agc ctg aag gcc tcg gac acc gcc atg tat tac tgt gcg aga ! 5-a# 49 ! VH6 15 cga ata acc atc aac cca gac aca tcc aag aac cag ttc tcc ctg cag ctg aac tct gtg act ccc gag gac acg gct gtg tat tac tgt L. gca aga ga ! 6-1# 50 , a 1, 1, 1 ! VH7 ţŌ cgg ttt gtc ttc tcc ttg gac acc tct gtc agc acg gca tat ctg Ų cag atc tgc agc cta aag gct gag gac act qcc qtq tat tac tqt gcg aga ga ! 74.1# 51 IJ <u>t</u>h

14

25

30

┙

Table 250: REdaptors, Extenders, and Bridges used for Cleavage and Capture of Human Heavy Chains in FR3.

A: HpyCH4V Probes of actual human HC genes

 $! \mbox{HpyCH4V}$ in FR3 of human HC, bases 35-56; only those with TGca site TGca;10,

RE recognition:tgca

of length 4 is expected at 10

1

6-1 agttctccctgcagctgaactc

```
3-11,3-07,3-21,3-72,3-48 cactgtatctgcaaatgaacag
          3
                                        3-09,3-43,3-20 ccctgtatctgcaaatgaacag
          4
                                                   5-51 ccgcctacctgcagtggagcag
          5
               3-15, 3-30, 3-30.5, 3-30.3, 3-74, 3-23, 3-33
                                                         cgctgtatctgcaaatgaacag
   5
          6
                                                  7-4.1 cggcatatctgcagatctgcag
          7
                                                   3-73 cggcgtatctgcaaatgaacag
          8
                                                    5-a ctgcctacctgcagtggagcag
          9
                                                   3-49
                                                         tcgcctatctgcaaatgaacag
  10
       B: HpyCH4V REdaptors, Extenders, and Bridges
        B.1 REdaptors
       ! Cutting HC lower strand:
       ! TmKeller for 100 mM NaCl, zero formamide
       ! Edapters for cleavage
                                                               T_m^W
                                                                              T_m^{K}
  15
       (ON HCFR36-1)
                            5'-agttctcccTGCAgctgaactc-3'
                                                               68.0
                                                                             64.5
       (ON HCFR36-1A)
                              5'-ttctcccTGCAgctgaactc-3'
                                                               62.0
                                                                             62.5
       (ON HCFR36-1B) ·
                              5'-ttctcccTGCAgctgaac-3'
                                                               56.0
                                                                             59.9
#16 F3 F3 #20
       (ON HCFR33-15)
                            5'-cgctgtatcTGCAaatgaacag-3'
                                                               64.0
                                                                             60.8
       (ON HCFR33-15A)
                              5'-ctgtatcTGCAaatgaacag-3'
                                                               56.0
                                                                             56.3
       (ON HCFR33-15B)
                              5'-ctgtatcTGCAaatgaac-3'
                                                               50.0
                                                                             53.1
       (ON HCFR33-11)
                            5'-cactgtatcTGCAaatgaacag-3'
                                                               62.0
                                                                             58.9
       (ON HCFR35-51)
                            5'-ccgcctaccTGCAgtggagcag-3'
                                                               74.0
                                                                            70.1
E
       B.2 Segment of synthetic 3-23 gene into which captured CDR3 is to be cloned
[]
_<u>:</u>25
                             XbaI...
       !D323*
               cgCttcacTaag tcT aga gac aaC tcT aag aaT acT ctC taC
$£
               scab..... designed gene 3-23 gene.....
١,]
D
            HpyCH4V
30
                               AflII...
            . . . .
            Ttg caG atg aac agc TtA aqG . . .
       B.3 Extender and Bridges
 35
       ! Extender (bottom strand):
       (ON HCHpyEx01)
                       5'-cAAgTAgAgAgTATTcTTAgAgTTgTc<u>TcTAgA</u>cTTAgTgAAgcg-3'
       ! ON_HCHpyEx01 is the reverse complement of
      ! 5'-cgCttcacTaag tcT aga gac aaC tcT aag aaT acT ctC taC Ttg -3'
 40
       ! Bridges (top strand, 9-base overlap):
```

2

```
!
                    (ON HCHpyBr016-1) 5'-cgCttcacTaag tcT aga gac aaC tcT aag-
                                                                   aaT acT ctC taC Ttg CAgctgaac-3' {3'-term C is blocked}
       5
                   ! 3-15 et al. + 3-11
                    (ON HCHpyBr023-15) 5'-cgCttcacTaag tcT aga gac aaC tcT aag-
                                                                   aaT acT ctC taC Ttg CAaatgaac-3' {3'-term C is blocked}
                   ! 5-51
     10
                   (ON_HCHpyBr045-51) 5'-cgCttcacTaag tcT aga gac aaC tcT aag-
                                                                   aaT acT ctC taC Ttg CAgtggagc-3' {3'-term C is blocked}
                   ! PCR primer (top strand)
     15
                   (ON HCHpyPCR)
                                                                        5'-cgCttcacTaag tcT aga gac-3'
4... P.4
                  C: BlpI Probes from human HC GLGs
[Ū
                                                              1-58,1-03,1-08,1-69,1-24,1-45,1-46,1-f,1-e acatggaGCTGAGCagcctgag
 L120
                         2
                                                                                                                                                             1-02 acatggaGCTGAGCaggctgag
The street street, str
                         3
                                                                                                                                                             1-18 acatggagctgaggagcctgag
                                                                                                                                                   5-51,5-a acctgcagtggagcagcctgaa
                         5
                                                                                                                        3-15,3-73,3-49,3-72 atctgcaaatgaacagcctgaa
                         6
                                                         3303,3-33,3-07,3-11,3-30,3-21,3-23,3305,3-48 atctgcaaatgaacagcctgag
7
                                                                                                                        3-20,3-74,3-09,3-43 atctgcaaatgaacagtctgag
                         8
                                                                                                                                                             74.1 atctgcagatctgcagcctaaa
                         9
                                                                                                                          3-66, 3-13, 3-53, 3-d atcttcaaatgaacagcctgag
4.4
                       10
                                                                                                                                                             3-64 atcttcaaatgggcagcctgag
 (]
                       11
                                     4301, 4-28, 4302, 4-04, 4304, 4-31, 4-34, 4-39, 4-59, 4-61, 4-b ccctgaaGCTGAGCtctgtgac
                      12
                                                                                                                                                               6-1 ccctgcagctgaactctgtgac
                       13
                                                                                                                                                2-70,2-05 tccttacaatgaccaacatgga
                      14
                                                                                                                                                            2-26 tccttaccatgaccaacatgga
                 D: BlpI REdaptors, Extenders, and Bridges
    35
                   D.1 REdaptors
                                                                                                                                                                         T_m^W
                                                                                                                                                                                                       T_mK
                  (BlpF3HC1-58)
                                                            5'-ac atg gaG CTG AGC agc ctg ag-3'
                                                                                                                                                                       70
                                                                                                                                                                                                    66.4
                  (BlpF3HC6-1)
                                                            5'-cc ctg aag ctg agc tct gtg ac-3'
                                                                                                                                                                       70
                                                                                                                                                                                                    66.4
                  ! BlpF3HC6-1 matches 4-30.1, not 6-1.
    40
```

D.2 Segment of synthetic 3-23 gene into which captured CDR3 is to be cloned

```
!
                                                                                     BlpI
                                XbaI...
        !D323*
                 cgCttcacTaag TCT AGA gac aaC tcT aag aaT acT ctC taC Ttg caG atg aac
   5
                               AflII...
                             agC TTA AGG
        D.3 Extender and Bridges
        ! Bridges
  10
        (BlpF3Br1) 5'-cgCttcacTcag tcT aga gaT aaC AGT aaA aaT acT TtG-
                            taC Ttg caG Ctg a GC agc ctg-3'
        (BlpF3Br2) 5'-cqCttcacTcaq tcT aga qaT aaC AGT aaA aaT acT TtG-
                            taC Ttg caG Ctg a|gc tct gtg-3'
                                                | lower strand is cut here
  15
        ! Extender
        (BlpF3Ext) 5'-
       TcAgcTgcAAgTAcAAAgTATTTTTAcTgTTATc<u>TcTAgA</u>cTgAgTgAAgcg-3'
        ! BlpF3Ext is the reverse complement of:
! 5'-cgCttcacTcag tcT aga gaT aaC AGT aaA aaT acT TtG taC Ttg caG Ctg a-3'
        (BlpF3PCR) 5'-cgCttcacTcag tcT aga gaT aaC-3'
       E: HpyCH4III Distinct GLG sequences surrounding site, bases 77-98
                        102#1,118#4,146#7,169#9,1e#10,311#17,353#30,404#37,4301 ccgtgtattactgtgcgagaga
          2
                        103#2,307#15,321#21,3303#24,333#26,348#28,364#31,366#32 ctgtgtattactgtgcgagaga
          3
                                                                      108#3 ccgtgtattactgtgcgagagg
          4
                                                                124#5,1f#11 ccgtgtattactgtgcaacaga
                                                                      145#6 ccatgtattactgtgcaagata
                                                                      158#8 ccgtgtattactgtgcggcaga
                                                                     205#12 ccacatattactgtgcacacag
          8
                                                                     226#13 ccacatattactgtgcacggat
          9
                                                                     270#14 ccacgtattactgtgcacggat
         10
                                                               309#16,343#27 ccttgtattactgtgcaaaaga
         11
                                                         313#18,374#35,61#50 ctgtgtattactgtgcaagaga
  35
         12
                                                                     315#19 ccgtgtattactgtaccacaga
         13
                                                                     320#20 ccttgtatcactgtgcgagaga
         14
                                                                     323#22 ccgtatattactgtgcgaaaga
         1.5
                                                              330#23,3305#25 ctgtgtattactgtgcgaaaga
         16
                                                                     349#29 ccgtgtattactgtactagaga
  40
         17
                                                                     372#33 ccgtgtattactgtgctagaga
         18
                                                                     373#34 ccgtgtattactgtactagaca
         19
                                                                      3d#36 ctgtgtattactgtaagaaaga
         20
                                                                     428#38 ccgtgtattactgtgcgagaaa
         21
                                                             4302#40,4304#41 ccgtgtattactgtgccagaga
  45
         22
                                                                     439#44 ctgtgtattactgtgcgagaca
```

L .

551#48 ccatgtattactgtgcgagaca

23

F: HpyCH4III REdaptors, Extenders, and Bridges F.1 REdaptors 5 ! ONs for cleavage of HC(lower) in FR3(bases 77-97) ! For cleavage with HpyCH4III, Bst4CI, or TaaI ! cleavage is in lower chain before base 88. 77 788 888 888 889 999 999 9 78 901 234 567 890 123 456 7 $T_m^{\ W}$ T_m^K 10 (H43.77.97.1-02#1) 5'-cc gtg tat tAC TGT gcg aga g-3' 64 62.6 (H43.77.97.1-03#2) 5'-c gtg tat tAC TGT gcg aga g-3' 60.6 62 (H43.77.97.108#3) 5'-cc gtg tat tAC TGT gcg aga g-3' 64 62.6 (H43.77.97.323#22) 5'-cc gt tat tac tgt gcg a g g-3' 58.7 60 (H43.77.97.330#23) 5'-ct gtg tat tac tgt gcg ama g-3' 60 58.7 15 5'-cm gtg tat tac tgt gcg aga g-3' (H43.77.97.439#44) 60.6 62 (H43.77.97.551#48) 5'-cc atg tat tac tgt gcg aga g-3' 62 60.6 (H43.77.97.5a#49) 5'-cc atg tat tAC TGT gcg aga 2-3' 58 58.3 C Ţ F.2 Extender and Bridges **120** ! XbaI and AflII sites in bridges are bunged IJ (H43.XABr1) 5'-ggtgtagtga-¥. [] IJ |TCT|AGt|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-|aac|agC|TTt|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat tgt gcg aga-3' ĮM (H43.XABr2) 5'-ggtgtagtga-2 [25 |TCT|AGt|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-=== ==== |aac|agC|TTt|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat tgt gcg aaa-3' 44 (H43.XAExt) 5'-ATAGTAGACT GCAGTGTCCT CAGCCCTTAA GCTGTTCATC TGCAAGTAGA-`.] Ü gAgTATTcTT AgAgTTgTcT cTAgATcAcT AcAcc-3' įż !H43.XAExt is the reverse complement of 30 ! 5'-qqtqtaqtqa-|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-|aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat -3' (H43.XAPCR) 5'-ggtgtagtga | TCT | AGA | gac | aac-3' 35 ! XbaI and AflII sites in bridges are bunged (H43.ABr1) 5'-ggtgtagtga-|aac|agC|TTt|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat tgt gcg aga-3' (H43.ABr2) 5'-ggtgtagtga-|aac|agC|TTt|AGq|gct|qaq|gac|aCT|GCA|Gtc|tac|tat tgt gcg aaa-3' 40 (H43.AExt) 5'-ATAGTAGACTGCAGTGTCCTCAGCCCTTAAGCTGTTTCACTACACC-3'

!(H43.AExt) is the reverse complement of 5'-ggtgtagtga! |aac|agC|TTA|AGg|qct|qaq|qac|aCT|GCA|Gtc|tac|tat -3'
(H43.APCR) 5'-ggtgtagtga |aac|agC|TTA|AGg|qct|q-3'

|aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgt gcg ag-3' |TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-|aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|t-3' (VHEx881) 5'-AATAGTAGAC TGCAGTGTCC TCAGCCCTTA AGCTGTTCAT CTGCAAGTAG-AGTAG-AGAAGTATCT TAGAGTTGTC TCTAGACTTA GTGAAGCG-3' ! note that VHEx881 is the reverse complement of the ON below Synthetic 3-23 as in Table 206 5'-cacatccgtg TrgTT cacggatgTg-3' [RC] 5'-cgCttcacTaag-5'-cgCttcacTaag-5'-cgCttcacTaag-Scab..... XbaI... (FOKJact) (VHBB881) (VHBA881) 25 35 30

|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-|aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgt Acg ag-3' (VH881PCR) 5'-cgCttcacTaag|TCT|AGA|gac|aac -3'

£dc dc£									ופבב	1101		aac	
A D	S I	ਬ		_		ອ	ď	Ö	Λ	T	Ð	Ð	
S\$ \$\$	12 43	t t	01	39	38	75	9٤	35	34	33	32	3.1	
								[ЯЗ				
		Ιτ	Mfe										
aga cca	וסבסובנ			lcgg	כבב								
166 101													
e e		ד ו	ō	Λ.	Э								
0E 6Z	8Z <i>L</i>	26 27	52	58	23								
		(82	-EV/L	(Dbđ	EKJ								
	• • • •	IobM		c									
			IMo	υM									
	660 0	ad tac	266	226	Ii			 					
67		ots 22											
9.6		M A		رغر 2	A	90	660	+5+	טָּדָט	- , 5			
	22	TZ 0	_	-	_								

```
Sites to be varied--->
                                   ***
            ----FR1----->|...CDR1......|---FR2-----
            46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
            A S G F T F S S Y A M S W V R
  5
            |gct|TCC|GGA|ttc|act|ttc|tct|tCG|TAC|Gct|atg|tct|tgg|gtt|cqC|
                                                                  143
            |cga|agg|cct|aag|tga|aag|aga|agc|atg|cga|tac|aga|acc|caa|gcg|
               BspEI
                                     BsiWI
                                                             |BstXI.
                             Sites to be varies---> ***
 10
            61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
             Q A P G K G L E W V S A I S G
           |CAa|qct|ccT|GGt|aaa|qqt|ttq|qaq|tqq|qtt|tct|qct|atc|tct|ggt|
                                                                  188
            |gtt|cga|gga|cca|ttt|cca|aac|ctc|acc|caa|aga|cga|tag|aqa|cca|
 15
        ...BstXI
           76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
 20
            S G G S T Y Y A D S V K G R F
           |tct|ggt|ggc|agt|act|tac|tat|qct|qac|tcc|gtt|aaa|qqt|cgc|ttc|
                                                                  233
           |aga|cca|ccg|tca|tga|atg|ata|cga|ctg|agg|caa|ttt|cca|gcg|aag|
            91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
T I S R D N S K N T L Y L Q M
 25
           |act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|
O
                                                                  278
           |tga|tag|aga|tct|ctg|ttg|aga|ttc|tta|tga|gag|atg|aac|gtc|tac|
1
                  | XbaI |
₫30
IJ
           ---FR3------>|
            106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
ų
            N S L R A E D T A V Y Y C A K
LU
           |aac|agC|TTA|AGg|qct|qag|qac|aCT|GCA|Gtc|tac|tat|tgc|gct|aaa|
                                                                  323
35
    . .
           | ttg|tcg|aat|tcc|cga|ctc|ctg|tga|cgt|cag|atg|ata|acg|cga|ttt|
th.
                |AflII |
                                    | PstI |
≅
             .....CDR3....................|----FR4------------------
Ü
            121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
= 40
            D Y E G T G Y A F D I W G Q G
ļ4
           |gac|tat|gaa|ggt|act|ggt|tat|gct|ttc|gaC|ATA|TGg|ggt|caa|ggt|
                                                                  368
           |ctg|ata|ctt|cca|tga|cca|ata|cga|aag|ctg|tat|acc|cca|gtt|cca|
٦.,
                                            | NdeI |
O
∔45
           136 137 138 139 140 141 142
            TMVTVSS
           |act|atG|GTC|ACC|gtc|tct|agt-
                                         389
           |tga|tac|cag|tgg|cag|aga|tca-
 50
                | BstEII |
                          143 144 145 146 147 148 149 150 151 152
                           A S T K G P S V F P
                          gcc tcc acc aaG GGC CCa tcg GTC TTC ccc-3'
 55
                          cgg agg tgq ttc ccg ggt agc cag aag ggg-5'
                                      Bsp120I.
                                               BbsI...(2/2)
                                      ApaI....
     (SFPRMET) 5'-ctg tct gaa cG GCC cag ccG-3'
 60
     (TOPFR1A) 5'-ctg tct gaa cG GCC cag ccG GCC atg gcc-
                gaa|gtt|CAA|TTG|tta|gag|tct|ggt|-
               |ggc|ggt|ctt|gtt|cag|cct|ggt|ggt|tct|tta-3'
     (BOTFR1B)
                        3'-caa|gtc|gga|cca|cca|aga|aat|gca|gaa|aga|acg|cga|-
               |cga|agg|cct|aag|tga|aag-5' | bottom strand
```

```
(BOTFR2)
                3'-acc|caa|gcg|-
                  |gtt|cga|gga|cca|ttt|cca|aac|ctc|acc|caa|aga|-5' ! bottom strand
      (BOTFR3)
                     a|cga|ctg|agg|caa|ttt|cca|gcg|aag|-
                  |tga|tag|aga|tct|ctg|ttg|aga|ttc|tta|tga|gag|atg|aac|gtc|tac|-
  5
              |ttg|tcg|aat|tcc|cga|ctc|ctg|tga-5'
      (F06)
                 5'-gC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgc|gct|aaa|-
             |gac|tat|gaa|ggt|act|ggt|tat|gct|ttc|gaC|ATA|TGg|ggt|c-3'
      (BOTFR4)
                3'-cga|aag|ctg|tat|acc|cca|gtt|cca|-
                  |tga|tac|cag|tgg|cag|aga|tca-
 10
                      cgg agg tgg ttc ccg ggt agc cag aag ggg-5' ! bottom strand
      (BOTPRCPRIM)
                            3'-gg ttc ccg ggt agc cag aag ggg-5'
     ! CDR1 diversity
 15
                 5'-|gct|TCC|GGA|ttc|act|ttc|tct|<1>|TAC|<1>|atq|<1>|-
     (ON-vgC1)
                                             CDR1.....6859
                    |tgg|gtt|cgC|CAa|gct|ccT|GG-3'
     !<1> stands for an equimolar mix of {ADEFGHIKLMNPQRSTVWY}; no C
 20
                                       (this is not a sequence)
     ! CDR2 diversity
                 5'-ggt|ttg|gag|tgg|gtt|tct|<2>|atc|<2>|<3>|-
     (ON-vgC2)
 25
                                           CDR2.....
                     |tct|ggt|ggc|<1>|act|<1>|tat|gct|gac|tcc|gtt|aaa|gg-3'
O
                     CDR2.....
     ! <1> is an equimolar mixture of {ADEFGHIKLMNPQRSTVWY}; no C
     ! <2> is an equimolar mixture of {YRWVGS}; no ACDEFHIKLMNPQT
<sup>[0</sup>30
     ! <3> is an equimolar mixture of {PS}; no ACDEFGHIKLMNQRTVWY
4.4
IJ
```

The true that the

14

ļļ

Table 800 (new)

The following list of enzymes was taken from http://rebase.neb.com/cgi-bin/asymmlist.

I have removed the enzymes that a) cut within the recognition, b) cut on both sides of the recognition, or c) have fewer than 2 bases between recognition and closest cut site.

REBASE Enzymes 04/13/2001

Type II restriction enzymes with asymmetric recognition sequences:

sequences	•		
Enzymes	Recognition Sequence	Isoschizomers	Suppliers
AarI	CACCTGCNNNN^NNNN_	_	У
AceIII	CAGCTCNNNNNNN^NNNN_	-	-
Bbr7I	GAAGACNNNNNNN^NNNN_	-	-
BbvI	GCAGCNNNNNNNN^NNNN_		У
BbvII	GAAGACNN^NNNN_		
Bce83I	CTTGAGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN	1 ~ -	_
BceAI	ACGGCNNNNNNNNNNNN^NN_	-	У
BcefI	ACGGCNNNNNNNNNNN^N_	_	-
BciVI	GTATCCNNNNN_N^	BfuI	У
BfiI	ACTGGGNNNN_N^	BmrI	У
BinI	GGATCNNNN^N_		
BscAI	GCATCNNNN^NN_	_	_
BseRI	GAGGAGNNNNNNNN_NN^	_	У
BsmFI	GGGACNNNNNNNNN\\\\\\\\\\\\\\\\\\\\\\\\\\	BspLU11III	У
${ t BspMI}$	ACCTGCNNNN^NNNN_	Acc36I	У
EciI	GGCGGANNNNNNNN NN^	_	У
Eco57I	CTGAAGNNNNNNNNNNNNNN NN	N^ BspKT5I	У
FauI	CCCGCNNNN^NN	BstFZ438I	У
FokI	GGATGNNNNNNNNN^NNNN	BstPZ418I	У
GsuI	CTGGAGNNNNNNNNNNNNN NN	1^ –	У
HgaI	GACGCNNNNN^NNNNN	_	У
HphI	GGTGANNNNNN N^	AsuHPI	У
MboII	GAAGANNNNNN N^	_	У
MlyI	GAGTCNNNNN^	SchI	У
MmeI	TCCRACNNNNNNNNNNNNNNNNN	IN NN^	
MnlI	CCTCNNNNN N^		У
PleI	$GAGTCNNNN^{\overline{N}}$	PpsI	У
RleAI	CCCACANNNNNNNN NNN^		_
SfaNI	GCATCNNNNN^NNNN	BspST5I	У
SspD5I	GGTGANNNNNNN^		_
Sth132I	CCCGNNNN^NNNN	_	-
StsI	GGATGNNNNNNNNNNNNNNN	-	_
TaqII	GACCGANNNNNNNN NN^, CA	ACCCANNNNNNNNN NN'	- -
Tth111II	CAARCANNNNNNNNNNNNN	-	_
UbaPI	CGAACG	-	-

The notation is $\hat{}$ means cut the upper strand and $\underline{}$ means cut the lower strand. If the upper and lower strand are cut at the same place, then only $\hat{}$ appears.

Table 120: MALIA3, annotated

! MALIA3 9532 bases

```
1 aat gct act act att agt aga att gat gcc acc ttt tca gct cgc gcc
 5
          gene ii continued
         49 cca aat gaa aat ata gct aaa cag gtt att gac cat ttg cga aat gta
         97 tct aat ggt caa act aaa tct act cgt tcg cag aat tgg gaa tca act
        145 gtt aca tgg aat gaa act tcc aga cac cgt act tta gtt gca tat tta
        193 aaa cat gtt gag cta cag cac cag att cag caa tta agc tct aag cca
10
        241 tcc gca aaa atg acc tct tat caa aag gag caa tta aag gta ctc tct
        289 aat cct gac ctg ttg gag ttt gct tcc ggt ctg gtt cgc ttt gaa gct
        337 cga att aaa acg cga tat ttg aag tct ttc ggg ctt cct ctt aat ctt
        385 ttt gat gca atc cgc ttt gct tct gac tat aat agt cag ggt aaa gac
        433 ctg att ttt gat tta tgg tca ttc tcg ttt tct gaa ctg ttt aaa gca
15
        481 ttt gag ggg gat tca ATG aat att tat gac gat tcc gca gta ttg gac
                RBS?....
                                Start gene x, ii continues
        529 gct atc cag tct aaa cat ttt act att acc ccc tct ggc aaa act tct
        577 ttt gca aaa gcc tct cgc tat ttt ggt ttt tat cgt cgt ctg gta aac
        625 gag ggt tat gat agt gtt gct ctt act atg cct cgt aat tcc ttt tgg
20
        673 cgt tat gta tct gca tta gtt gaa tgt ggt att cct aaa tct caa ctg
        721 atg aat ctt tct acc tqt aat aat qtt qtt ccq tta qtt cqt ttt att
        769 aac gta gat ttt tct tcc caa cgt cct gac tgg tat aat gag cca gtt
        817 ctt aaa atc gca TAA
                            End X & II
25
        832 ggtaattca ca
            M1
                             E5
                                                Q10
                                                                     T15
        843 ATG att aaa gtt gaa att aaa cca tct caa gcc caa ttt act act cgt
            Start gene V
30
     !
     !
            S17
                        S20
                                            P25
                                                                 E30
        891 tct ggt gtt tct cgt cag ggc aag cct tat tca ctg aat gag cag ctt
     !
                    V35
                                        E40
35
        939 tgt tac gtt gat ttg ggt aat gaa tat ccg gtt ctt gtc aag att act
                D50
                                    A55
                                                        L60
        987 ctt gat gaa ggt cag cca gcc tat gcg cct ggt cTG TAC Acc gtt cat
                                                         BsrGI...
```

```
! L65
                                 V70
                                                    S75
                                                                        R80
         1035 ctg tcc tct ttc aaa gtt ggt cag ttc ggt tcc ctt atg att gac cgt
                             P85
                                     K87 end of V
    5
        1083 ctg cgc ctc gtt ccg gct aag TAA C
         1108 ATG gag cag gtc gcg gat ttc gac aca att tat cag gcg atg
              Start gene VII
   10
         1150 ata caa atc tcc gtt gta ctt tgt ttc gcg ctt ggt ata atc
                               VII and IX overlap.
                               ..... S2 V3 L4 V5
                                                                   S10
         1192 gct ggg ggt caa agA TGA gt gtt tta gtg tat tct ttc gcc tct ttc gtt
   15
                                 End VII
                              start IX
G20
              L13
                  W15
                                                            T25
                                                                           E29
1,1
         1242 tta ggt tgg tgc ctt cgt agt ggc att acg tat ttt acc cgt tta atg gaa
(O
U
   20
         1293 act tcc tc
IJ
t3
t i
              .... stop of IX, IX and VIII overlap by four bases
         1301 ATG aaa aag tot tta gto cto aaa goo tot gta goo gtt got acc cto
73
              Start signal sequence of viii.
  25
1349 gtt ccg atg ctg tct ttc gct gat gat gat gat ccc gca aaa gcg
Ü
                                         mature VIII --->
         1397 gcc ttt aac tcc ctg caa gcc tca gcg acc gaa tat atc ggt tat gcg
         1445 tgg gcg atg gtt gtt gtc att
   30
         1466 gtc ggc gca act atc ggt atc aag ctg ttt aag
         1499 aaa ttc acc tcg aaa gca ! 1515
              ...... -35 ..
         agc tga taaaccgat acaattaaag gctccttttg
   35
                            .... -10 ...
         1552 gagccttttt ttttGGAGAt ttt ! S.D. underlined
                   <---->
III signal sequence ---->>
```

		!			М	к	к	L	L	F	А	I	P	L	v					
			1575	caac	GTO	G aaa	a aa	a tta	a tta	a tt	c gca	a att	t cct	tt	a gt	t!	1611			
		!																		
	_	!		V	P	F	Y	S	H	S	Α	Q								
	5		1612	gtt	cct	ttc	tat	tct	cac		•	-	tCT							
		!								Apa	aLI.	• •								
		!	1640		am a	ama		a. a			max	ama	m em	~~~		aas .			-	
			1642					CAG												
	10							ATC	TCC	TGC	ACT	تاتاتا	AGC	AGC	TCC	AAC	ATC	GGG	GCA	
	10	!	1720			stEI:		CMA	CAC	mcc.	ma c	C D C	C	C III III	CCN	CCT	202	ccc	000	
			1729 1777					GTA TAT												
			1825					TCC												CGA
			1870					GCT							100	CIG	GCC	AIC	ACI	
	15		1900					TCC												
			1930					GTC							GTC	ACC	GTC			
71		!												B	stEI	Ι				
C			1969		CTA	GGT	CAG	CCC	AAG	GCC	AAC	CCC	ACT							
LJ			2002		CTG	TTC	CCG	CCC	TCC	TCT	GAG	GAG	CTC	CAA	GCC	AAC	AAG	GCC	ACA	CTA
from Arra	20		2050		GTG	TGT	CTG	ATC	AGT	GAC	TTC	TAĊ	CCG	GGA	GCT	GTG	ACA	GTG	GCC	TGG
O			2098		AAG	GCA	GAT	AGC	AGC	CCC	GTC	AAG	GCG	GGA	GTG	GAG	ACC	ACC	ACA	CCC
M			2146		TCC	AAA	CAA	AGC	AAC	AAC	AAG	TAC	GCG	GCC	AGC	AGC	TAT	CTG	AGC	CTG
11			2194		ACG	CCT	GAG	CAG	TGG	AAG	TCC	CAC	AGA	AGC	TAC	AGC	TGC	CAG	GTC	ACG
∓. ##.			2242		CAT	GAA	GGG	AGC	ACC	GTG	GAG	AAG	ACA	GTG	GCC	CCT	ACA	GAA	TGT	TCA
#4	25		2290		TAA	TAA	ACC	G CCI	CCA	CCG <u>G</u>	GCG	CGCC/	TAF	CTA	TTC	AA G	GAGA	CAGT	ATA	Ą
17.4 15.41 df		!								A	scI.									
14		!																		
		!					-												>	
	20	!	0040		M			L											L	
	30		2343		ATG	AAA	TAC	CTA	TTG	CCT	ACG	GCA	GCC	GCT	GGA	TTG	TTA	TTA	CTC	
		:			16	17	10	19	20		21	22								
		1						P			M									
		٠	2388					ccG (
	35	!		9																
		!						oMI		/ 2)										
		!																		
																				*

!

```
FR1 (DP47/V3-23) -----
                               23 24 25 26 27 28 29 30
                                 VQLL
                                            E
       2409
                               gaa|gtt|CAA|TTG|tta|gag|tct|ggt|
  5
                                   | MfeI |
           -----FR1-------
           31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
            GGLVQP
                             G G S L R
  10
     2433 |ggc|ggt|ctt|gtt|cag|cct|ggt|ggt|tct|tta|cgt|ctt|tct|tgc|gct|
           ----FR1----->|...CDR1.....|---FR2-----
           46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
            A S G F T F S S Y A M
  15
      2478 |gct|TCC|GGA|ttc|act|ttc|tct|tCG|TAC|Gct|atg|tct|tgg|gtt|cgC|
             | BspEI |
                                | BsiWI|
                                                    |BstXI.
-----FR2-------|...CDR2......
Ļij
           61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
  20
            Q A P G K G L E W V
                                        S
                                           Α
       2523 | CAa|gct|ccT|GGt|aaa|ggt|ttg|gag|tgg|gtt|tct|gct|atc|tct|ggt|
IJ
Įñ,
     ! ...BstXI
                    - 1
[]
          25
           76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
           S G G S T Y Y A D S V K
      2568 |tct|ggt|ggc|agt|act|tac|tat|gct|gac|tcc|gtt|aaa|ggt|cgc|ttc|
  30
          -----FR3-----
            91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
               I S R
                       D
                          N
                             S
                                K
                                        L
                                           Y
                                   N
                                     T
      2613 |act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|
                | XbaI |
  35
           ---FR3------>|
           106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
                  L R A E D T A V Y Y
      2658 | aac|aqC|TTA|AGq|qct|qaq|qac|aCT|GCA|Gtc|tac|tat|tqc|qct|aaa|
```

```
ţ
                      |AflII |
                                            | PstI |
        !
                121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
    5
                                     G
                                        Y
                                            Α
                                                F
                                                    D
                                                        I
                |gac|tat|gaa|ggt|act|ggt|tat|gct|ttc|gaC|ATA|TGg|ggt|caa|ggt|
                                                     | NdeI | (1/4)
               10
                136 137 138 139 140 141 142
                     М
                         V
                             Т
                                 V
                                     S
          2748 |act|atG|GTC|ACC|qtc|tct|aqt
                      | BstEII |
        ! From BstEII onwards, pV323 is same as pCES1, except as noted.
   15
        ! BstEII sites may occur in light chains; not likely to be unique in final
        ! vector.
11
den eine erne
                                 143 144 145 146 147 148 149 150 151 152
                                             K
                                                 G
                                                     P
                                 Α
                                      S
   20
          2769
                                 gcc tcc acc aaG GGC CCa tcg GTC TTC ccc
1344
                                              Bsp120I.
                                                            BbsI...(2/2)
Įħ
                                              ApaI....
1.0
              153 154 155 156 157 158 159 160 161 162 163 164 165 166 167
   25
                           S
                               s
                                   K
                                       S
                                          Т
                                              S
                                                  G
2799 ctg gca ccC TCC TCc aag agc acc tct ggg ggc aca gcg gcc ctg
[]
                        BseRI...(2/2)
                168 169 170 171 172 173 174 175 176 177 178 179 180 181 182
   30
                         L
                             ν
                                 K
                                    D
                                        Y
                                            F
                                                Р
                                                    Ε
                                                        Ρ
          2844
                ggc tgc ctg GTC AAG GAC TAC TTC CCc gaA CCG GTg acg gtg tcg
                                                     AgeI....
                183 184 185 186 187 188 189 190 191 192 193 194 195 196 197
   35
                             G
                                 Α
                                    L
                                        T
                                            S
                                                G
                                                        Н
                tgg aac tca GGC GCC ctg acc agc ggc gtc cac acc ttc ccg gct
                            KasI...(1/4)
                198 199 200 201 202 203 204 205 206 207 208 209 210 211 212
```

.

		!	v	L Q	s	s	G	L	Y	S	L	S	s	V	V	T	
		2934	gtc c	ta cag	tCt	agc	GGa c	tc t	ac 1	tcc	ctc	agc	agc	gta	gtg	acc	
		!			(Bsu	361.)(k	nock	ced o	out)							
		!															
	5	!	213 2	14 215	216	217	218 2	19 2	220 2	221	222	223	224	225	226	227	
		!	v	P S	s	s	L	G	T	Q	T	Y	I	С	N	v	
		2979	gtg c	cC tCt	tct	agc	tTG G	gc a	acc o	cag	acc	tac	atc	tgc	aac	gtg	
		!		(Bs	tXI) N . E	3. de	estr	ucti	on o	of Bs	stXI	& Br	omI s	ites.
		!															
	<i>10</i>	!	228 2	29 230	231	232	233 2	34 2	235 2	236	237	238	239	240	241	242	
		!	N	н к	P	s	N	T	K	V	D	ĸ	K	v	E	P	
		3024	aat c	ac aag	ccc	agc .	aac a	cc a	ag q	gtg	gac	aag	aaa	gtt	gag	ccc	
		!															
		!	243 2	44 245													
	<i>15</i>	!	K	s c	Α	Α	A	Н	Н	H	Н	H	H	s	Α		
2 EL		3069	aaa t	ct tgt	GCG	GCC	GCt c	at c	ac d	cac	cat	cat	cac	tct	gct		
		!			NotI		• •										
Į.		!															
erne gregamen greg ett		!	E	Q K	L	I	S	E	E	D	L	N	G.	A	A		
14	20	3111	gaa c	aa aaa	ctc	atc	tca g	aa g	gag q	gat	ctg	aat	ggt	gcc	gca		
17		!															
ťΠ	<u>(</u> ^																
: ₽=4		!	D	I N	D	D	R	M	Α	S	G	; <i>P</i>	.				
		3153	GAT A	TC aac	gat	gat	cgt a	tg	gct	. AG	C g	igc g	cc				
łå	25	! rEK cleavage site NheI KasI															
÷		!	EcoRV	• •													
13		ŗ															
# ····		! Domain	n 1														
		!	Α	E	T V	E	S	С	L	A	•						
	30	3183	gct	gaa a	ct gt	t gaa	a agt	tgt	tta	a gc	a						
		!															
		!															
		!	K P	H	T E	I	S	F									
		3210 a	aaa ccc	cat a	ca ga	a aa	t tca	ttt									
	35	!															
		i	T N	V	W K	D	D	K	T								
		3234 8	aCT AAC	GTC T	gg aa	A GA	C GAC	AAA	ACt	:							
		!															
		!	L D	R	Y A	N	Y	E	G	С	I	. W	N	ī Æ	I A	. G	V

```
3261 tta gat cgt tac gct aac tat gag ggt tgt ctg tgG AAT GCt aca ggc gtt
                                                            BsmI
                                                           G
    5
          3312 gta gtt tgt act ggt GAC GAA ACT CAG TGT TAC GGT ACA TGG GTT cct att
                       Α
                           Ι
          3363 ggg ctt gct atc cct gaa aat
   10
        ! L1 linker ----
               Ε
                       G
                           G
                               S
                                   Ε
                                       G
         3384 gag ggt ggt ggc tct gag ggt ggc ggt tct
               Ε
                   G
                       G
   15
         3414 gag ggt ggc ggt tct gag ggt ggc ggt act
[]
        ! Domain 2 -----
13
         3444 aaa cct cct gag tac ggt gat aca cct att ccg ggc tat act tat atc aac
to
Ų
         3495 cct ctc gac ggc act tat ccg cct ggt act gag caa aac ccc gct aat cct
  20
         3546 aat cct tct ctt GAG GAG tct cag cct ctt aat act ttc atg ttt cag aat
LJ
                              BseRI
C
Ī
         3597 aat agg ttc cga aat agg cag ggg gca tta act gtt tat acg ggc act
         3645 gtt act caa ggc act gac ccc gtt aaa act tat tac cag tac act cct
3693 gta tca tca aaa gcc atg tat gac gct tac tgg aac ggt aaa ttC AGA
  25
+4
         3741 GAC TGc gct ttc cat tct ggc ttt aat gaa gat cca ttc gtt tgt gaa
IJ
               AlwNI
         3789 tat caa ggc caa tcg tct gac ctg cct caa cct cct gtc aat gct
  30
         3834 ggc ggc ggc tct
        ! start L2 -----
         3846 ggt ggt ggt tct
         3858 ggt ggc ggc tct
         3870 gag ggt ggt ggc tct gag ggt ggc ggt tct
  35
         3900 gag ggt ggc ggc tct gag gga ggc ggt tcc
         3930 ggt ggt ggc tct ggt
                                     ! end L2
       ! Domain 3 ---
                       D
                         F D Y E
```

K

М

Α

N

Α

N

K

3945 tcc ggt gat ttt gat tat gaa aag atg gca aac gct aat aag ggg gct ! M N Α D E N Α L S D 3993 atg acc gaa aat gcc gat gaa aac gcg cta cag tct gac gct aaa ggc 5 K D V Α D Y G Α Α Ι 4041 aaa ett gat tet gte get aet gat tae ggt get get ate gat ggt_tte I G L A N G D V S G N G A T 10 4089 att ggt gac gtt tcc ggc ctt gct aat ggt aat ggt gct act ggt gat F Α G S S N Q M Α V 4137 ttt gct ggc tct aat tcc caa atg gct caa gtc ggt gac ggt gat aat 15 S L М N N F R Q Y L Ρ S L 4185 tca cct tta atg aat aat ttc cgt caa tat tta cct tcc ctc cca S V E С Α R P F V F S G K Ε Р Y 4233 tog gtt gaa tgt cgc cct ttt gtc ttt agc gct ggt aaa cca tat gaa 20 F S Ι D С D K Ι 4281 ttt tct att gat tgt gac aaa ata aac tta ttc cgt End Domain 3 25 G V F A F L L Y V A T F M 4317 ggt gtc ttt gcg ttt ctt tta tat gtt gcc acc ttt atg tat gta ttt start transmembrane segment Т Α F N 30 4365 tct acg ttt gct aac ata ctg R Ν K Ε 4386 cgt aat aag gag tct TAA ! stop of iii Intracellular anchor. 35 M1 P2 V L L5 L L10 L G I P R F L G15 4404 to ATG cca gtt ctt ttg ggt att ccg tta tta ttg cgt ttc ctc ggt Start VI

4451 ttc ctt ctg gta act ttg ttc ggc tat ctg ctt act ttt ctt aaa aag 4499 ggc ttc ggt aag ata gct att gct att tca ttg ttt ctt gct ctt att 4547 att ggg ctt aac tca att ctt gtg ggt tat ctc tct gat att agc gct 4595 caa tta ccc tct gac ttt gtt cag ggt gtt cag tta att ctc ccg tct 5 4643 aat gcg ctt ccc tgt ttt tat gtt att ctc tct gta aag gct gct att 4691 ttc att ttt gac gtt aaa caa aaa atc gtt tct tat ttg gat tgg gat ! M1 A2 V3 F5 L10 G13 4739 aaa TAA t ATG gct gtt tat ttt gta act ggc aaa tta ggc tct gga 10 end VI Start gene I 14 15 16 17 18 19 20 21 22 23 24 25 26 V S G K Ι Q D K Ι V Α 4785 aag acg ctc gtt agc gtt ggt aag att cag gat aaa att gta gct 15 29 30 31 32 33 34 35 36 37 38 39 42 43 40 41 G С K Ι Α L 4830 ggg tgc aaa ata gca act aat ctt gat tta agg ctt caa aac ctc 20 45 44 46 47 48 49 50 51 52 53 54 55 56 57 58 ₽ Q V G F Α K Т Р R ν Ι R 4875 ccg caa gtc ggg agg ttc gct aaa acg cct cgc gtt ctt aga ata 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 25 Ρ D P S Ι S D Ι L L 4920 ccg gat aag cct tct ata tct gat ttg ctt gct att ggg cgc ggt 74 75 76 77 78 79 80 81 82 83 85 86 84 87 88 N D S Y D Ε G D N K N L 30 4965 aat gat tcc tac gat gaa aat aaa aac ggc ttg ctt gtt ctc gat 90 91 92 89 93 94 95 96 97 98 99 100 101 102 103 С G W F N Т R S K 5010 gag tgc ggt act tgg ttt aat acc cgt tct tgg aat gat aag gaa 35 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 R Q Ρ Ι Ι D L Η R K L G 5055 aga cag ccg att att gat tgg ttt cta cat gct cgt aaa tta gga

119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 L V L Ι I F Q D S · I 5100 tgg gat att att ttt ctt gtt cag gac tta tct att gtt gat aaa 5 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 R s Α L Α Ε Н V Y 5145 cag gcg cgt tct gca tta gct gaa cat gtt gtt tat tgt cgt cgt 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 10 L D Ι Ρ F V R T L G T L Y 5190 ctg gac aga att act tta cct ttt gtc ggt act tta tat tct ctt 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 Т G S K М Ρ L Р K L Н V V 15 5235 att act ggc tcg aaa atg cct ctg cct aaa tta cat gtt ggc gtt ! 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 L V K Y G D S Q S Р 5280 gtt aaa tat ggc gat tct caa tta agc cct act gtt gag cgt tgg 20 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 Y Т G K N L Y N Α Y D 5325 ctt tat act ggt aag aat ttg tat aac gca tat gat act aaa cag 25 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 S S Y D S G F N V Y 5370 gct ttt tct agt aat tat gat tcc ggt gtt tat tct tat tta acg 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 30 Y L S H G R Y F K P L 5415 cct tat tta tca cac ggt cgg tat ttc aaa cca tta aat tta ggt 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 K M K \mathbf{L} \mathbf{T} K Ι Y L K K 35 5460 cag aag atg aaa tta act aaa ata tat ttg aaa aag ttt tct cgc 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 C L Α I G F A S Α F 5505 gtt ctt tgt ctt gcg att gga ttt gca tca gca ttt aca tat agt

June 11.

! 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 P K P Ε K 5550 tat ata acc caa cct aag ccg gag gtt aaa aag gta gtc tct cag 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 F Ι Т Y D D K F Т D 5595 acc tat gat ttt gat aaa ttc act att gac tct tct cag cgt ctt 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 N L S Y R Y V F K 5640 aat cta agc tat cgc tat gtt ttc aag gat tct aag gga aaa TTA PacI 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 Ι N S D D L Q K Q G Y S L Y 5685 ATT AAt agc gac gat tta cag aag caa ggt tat tca ctc aca tat PacI 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 i I D L С Т V S I K K G N S N E iv M1 K 5730 att gat tta tgt act gtt tcc att aaa aaa ggt aat tca aAT Gaa Start IV 344 345 346 347 348 349 V С i Ι K N .End of I iv L3 L N5 V 17 N F V10 5775 att gtt aaa tgt aat TAA T TTT GTT ! IV continued.... 5800 ttc ttg atg ttt gtt tca tca tct tct ttt gct cag gta att gaa atg 5848 aat aat tog cot otg ogc gat ttt gta act tgg tat toa aag caa toa 5896 ggc gaa tcc gtt att gtt tct ccc gat gta aaa ggt act gtt act gta 5944 tat tca tct gac gtt aaa cct gaa aat cta cgc aat ttc ttt att tct 5992 gtt tta cgt gct aat aat ttt gat atg gtt ggt tca att cct tcc ata 6040 att cag aag tat aat cca aac aat cag gat tat att gat gaa ttg cca 6088 tca tct gat aat cag gaa tat gat gat aat tcc gct cct tct ggt ggt 6136 ttc ttt gtt ccg caa aat gat aat gtt act caa act ttt aaa att aat 6184 aac gtt cgg gca aag gat tta ata cga gtt gtc gaa ttg ttt gta aag

```
6232 tct aat act tct aaa tcc tca aat qta tta tct att gac ggc tct aat
       6280 cta tta qtt qtt TCT qca cct aaa qat att tta gat aac ctt cct caa
                             ApaLI removed
       6328 ttc ctt tct act gtt gat ttg cca act gac cag ata ttg att gag ggt
 5
       6376 ttg ata ttt gag gtt cag caa ggt gat gct tta gat ttt tca ttt gct
       6424 gct ggc tct cag cgt ggc act gtt gca ggc ggt gtt aat act gac cgc
       6472 ctc acc tct gtt tta tct tct gct ggt ggt tcg ttc ggt att ttt aat
       6520 ggc gat gtt tta ggg cta tca gtt cgc gca tta aag act aat agc cat
       6568 tca aaa ata ttg tct gtg cca cgt att ctt acg ctt tca ggt cag aag
10
       6616 ggt tot ato tot gtT GGC CAg aat qtc cot ttt att act ggt cgt gtq
     !
                              MscI
       6664 act ggt gaa tot goo aat gta aat coa ttt cag acg att gag cgt
       6712 caa aat gta ggt att tcc atg agc gtt ttt cct gtt gca atg gct ggc
       6760 ggt aat att gtt ctg gat att acc agc aag gcc gat agt ttg agt tct
15
       6808 tet act cag gea agt gat gtt att act aat caa aga agt att get aca
       6856 acg gtt aat ttg cgt gat gga cag act ctt tta ctc ggt ggc ctc act
       6904 gat tat aaa aac act tct caa gat tct ggc gta ccg ttc ctg tct aaa
       6952 atc cct tta atc ggc ctc ctg ttt agc tcc cgc tct gat tcc aac gag
       7000 gaa age acg tta tae gtg etc gte aaa gea ace ata gta ege gee etg
20
       7048 TAG cggcgcatt
            End IV
       7060 aagegeggeg ggtgtggtgg ttaegegeag egtgaeeget acaettgeea gegeeetage
       7120 geoegeteet ttegetttet teeetteett tetegeeaeg tteGCCGGCt tteeeegtea
                                                           NgoMI
25
       7180 agetetaaat egggggetee etttagggtt eegatttagt getttaegge acetegaece
       7240 caaaaaactt gatttgggtg atggttCACG TAGTGggcca tcgccctgat agacggtttt
                                        DraIII
       7300 tcgccctttG ACGTTGGAGT Ccacgttctt taatagtgga ctcttgttcc aaactggaac
                     DrdI
30
       7360 aacactcaac cctatctcgg gctattcttt tgatttataa gggattttgc cgatttcgga
       7420 accaccatca aacaggattt tegeetgetg gggcaaacca gegtggaceg ettgetgeaa
       7480 ctctctcagg gccaggcggt gaaqqqcaat CAGCTGttqc cCGTCTCact gqtqaaaaqa
                                             PvuII.
                                                         BsmBI.
       7540 aaaaccaccc tGGATCC AAGCTT
35
                                HindIII (1/2)
                        BamHI
                        Insert carrying bla gene
               gcaggtg gcacttttcg gggaaatgtg cgcggaaccc
       7600 ctatttgttt atttttctaa atacattcaa atatGTATCC gctcatgaga caataaccct
                                                 BciVI
```

```
7660 gataaatgct tcaataatat tgaaaaAGGA AGAgt
        !
                                           RBS.?...
               Start bla gene
         7695 ATG agt att caa cat ttc cgt gtc gcc ctt att ccc ttt ttt gcg gca ttt
   5
         7746 tgc ctt cct gtt ttt gct cac cca gaa acg ctg gtg aaa gta aaa gat gct
         7797 gaa gat cag ttg ggC gCA CGA Gtg ggt tac atc gaa ctg gat ctc aac agc
                                    BssSI...
                                ApaLI removed
         7848 ggt aag atc ctt gag agt ttt cgc ccc gaa gaa cgt ttt cca atg atg agc
  10
         7899 act ttt aaa gtt ctg cta tgt cat aca cta tta tcc cgt att gac gcc ggg
         7950 caa gaG CAA CTC GGT CGc cgg gcg cgg tat tct cag aat gac ttg gtt gAG
         8001 TAC Tca cca gtc aca gaa aag cat ctt acg gat ggc atg aca gta aga gaa
              ScaI
  15
         8052 tta tgc agt gct gcc ata acc atg agt gat aac act gcg gcc aac tta ctt
         8103 ctg aca aCG ATC Gga gga ccg aag gaq cta acc qct ttt ttg cac aac atg
7
                       PvuI
1
         8154 ggg gat cat gta act cgc ctt gat cgt tgg gaa ccg gag ctg aat gaa gcc
ťÖ.
Ų
         8205 ata cca aac gac gag cgt gac acc acg atg cct gta gca atg cca aca acg
  20
         8256 tTG CGC Aaa cta tta act ggc gaa cta ctt act cta gct tcc cgg caa caa
Ų
               FspI....
O
ĬÑ
≘
         8307 tta ata gac tgg atg gag gcg gat aaa gtt gca gga cca ctt ctg cgc tcg
8358 GCC ctt ccG GCt ggc tgg ttt att gct gat aaa tct gga gcc ggt gag cgt
  25
1
         8409 gGG TCT Cgc ggt atc att gca gca ctg ggg cca gat ggt aag ccc tcc cgt
IJ
ļ.A.
         8460 atc gta gtt atc tac acG ACg ggg aGT Cag gca act atg gat gaa cga aat
                                     AhdI
  30
         8511 aga cag atc gct gag ata ggt gcc tca ctg att aag cat tgg TAA ctgt
         8560 cagaccaagt ttactcatat atactttaga ttgatttaaa acttcatttt taatttaaaa
         8620 ggatctaggt gaagatcctt tttgataatc tcatgaccaa aatcccttaa cgtgagtttt
         8680 cgttccactg tacgtaagac cccc
  35
         8704 AAGCTT
                       GTCGAC tgaa tggcgaatgg cgctttgcct
              HindIII SalI..
              (2/2)
                       HincII
         8740 ggtttccggc accagaagcg gtgccggaaa gctggctgga gtgcgatctt
```

8790 CCTGAGG Bsu36I 8797 ccgat actgtcgtcg tcccctcaaa ctggcagatg 8832 cacggttacg atgcgcccat ctacaccaac gtaacctatc ccattacggt caatccgccg 5 8892 tttgttccca cggagaatcc gacgggttgt tactcgctca catttaatgt tgatgaaagc 8952 tggctacagg aaggccagac gcgaattatt tttgatggcg ttcctattgg ttaaaaaatg 9012 agctgattta acaaaaattt aacgcgaatt ttaacaaaat attaacgttt acaATTTAAA 9072 Tatttgctta tacaatcttc ctgtttttgg ggcttttctg attatcaacc GGGGTAcat 10 RBS? 9131 ATG att gac atg cta gtt tta cga tta ccg ttc atc gat tct ctt gtt tgc Start gene II 9182 tcc aga ctc tca ggc aat gac ctg ata gcc ttt gtA GAT CTc tca aaa ata BqlII... 15 9233 gct acc ctc tcc ggc atg aat tta tca gct aga acg gtt gaa tat cat att 9284 gat ggt gat ttg act gtc tcc ggc ctt tct cac cct ttt gaa tct tta cct 9335 aca cat tac tca ggc att gca ttt aaa ata tat gag ggt tct aaa aat ttt 9386 tat cct tgc gtt gaa ata aag gct tct ccc gca aaa gta tta cag ggt cat 9437 aat gtt ttt ggt aca acc gat tta gct tta tgc tct gag gct tta ttg ctt 20 9488 aat ttt gct aat tct ttg cct tgc ctg tat gat tta ttg gat gtt ! 9532 ! gene II continues

Table 120B: Sequence of MALIA3, condensed

	LOCUS		MALIA3	9532	C	IRCULAR		
	ORIGIN							
		1	AATGCTACTA	CTATTAGTAG	AATTGATGCC	ACCTTTTCAG	CTCGCGCCCC	AAATGAAAAT
5		61	ATAGCTAAAC	AGGTTATTGA	CCATTTGCGA	AATGTATCTA	ATGGTCAAAC	TAAATCTACT
		121	CGTTCGCAGA	Α ΤΤΙΚΕ Α ΑΤΙ	ΔΔΟΤΩΤΤΔΟΔ	ТССДДТСДДД	СТТССАСАСА	СССТАСТТТА

	_	ARIGCIACIA	CIAIIAGIAG	ANTIGNIGEE	ACCITICAG	CICGCGCCCC	MAN GAMAN
5	61	ATAGCTAAAC	AGGTTATTGA	CCATTTGCGA	AATGTATCTA	ATGGTCAAAC	TAAATCTACT
	121	CGTTCGCAGA	ATTGGGAATC	AACTGTTACA	TGGAATGAAA	CTTCCAGACA	CCGTACTTTA
	181	GTTGCATATT	TAAAACATGT	TGAGCTACAG	CACCAGATTC	AGCAATTAAG	CTCTAAGCCA
	241	TCCGCAAAAA	TGACCTCTTA	TCAAAAGGAG	CAATTAAAGG	TACTCTCTAA	TCCTGACCTG
	301	TTGGAGTTTG	CTTCCGGTCT	GGTTCGCTTT	GAAGCTCGAA	TTAAAACGCG	ATATTTGAAG
10	361	TCTTTCGGGC	TTCCTCTTAA	TCTTTTTGAT	GCAATCCGCT	TTGCTTCTGA	CTATAATAGT
	421	CAGGGTAAAG	ACCTGATTTT	TGATTTATGG	TCATTCTCGT	TTTCTGAACT	GTTTAAAGCA
	481	TTTGAGGGGG	ATTCAATGAA	TATTTATGAC	GATTCCGCAG	TATTGGACGC	TATCCAGTCT
	541	AAACATTTTA	CTATTACCCC	CTCTGGCAAA	ACTTCTTTTG	CAAAAGCCTC	TCGCTATTTT
	601	GGTTTTTATC	GTCGTCTGGT	AAACGAGGGT	TATGATAGTG	TTGCTCTTAC	TATGCCTCGT
15	661	AATTCCTTTT	GGCGTTATGT	ATCTGCATTA	GTTGAATGTG	GTATTCCTAA	ATCTCAACTG
	721	ATGAATCTTT	CTACCTGTAA	TAATGTTGTT	CCGTTAGTTC	GTTTTATTAA	CGTAGATTTT
	781	TCTTCCCAAC	GTCCTGACTG	GTATAATGAG	CCAGTTCTTA	AAATCGCATA	AGGTAATTCA
	841	CAATGATTAA	AGTTGAAATT	AAACCATCTC	AAGCCCAATT	TACTACTCGT	TCTGGTGTTT
	901	CTCGTCAGGG	CAAGCCTTAT	TCACTGAATG	AGCAGCTTTG	TTACGTTGAT	TTGGGTAATG
<i>20</i>	961	AATATCCGGT	TCTTGTCAAG	ATTACTCTTG	ATGAAGGTCA	GCCAGCCTAT	GCGCCTGGTC
	1021	TGTACACCGT	TCATCTGTCC	TCTTTCAAAG	TTGGTCAGTT	CGGTTCCCTT	ATGATTGACC
	1081	GTCTGCGCCT	CGTTCCGGCT	AAGTAACATG	GAGCAGGTCG	CGGATTTCGA	CACAATTTAT
	1141	CAGGCGATGA	TACAAATCTC	CGTTGTACTT	TGTTTCGCGC	TTGGTATAAT	CGCTGGGGGT
	1201	CAAAGATGAG	TGTTTTAGTG	TATTCTTTCG	CCTCTTTCGT	TTTAGGTTGG	TGCCTTCGTA
25	1261	GTGGCATTAC	GTATTTTACC	CGTTTAATGG	AAACTTCCTC	ATGAAAAAGT	CTTTAGTCCT
	1321	CAAAGCCTCT	GTAGCCGTTG	CTACCCTCGT	TCCGATGCTG	TCTTTCGCTG	CTGAGGGTGA
	1381	CGATCCCGCA	AAAGCGGCCT	TTAACTCCCT	GCAAGCCTCA	GCGACCGAAT	ATATCGGTTA
	1441	TGCGTGGGCG	ATGGTTGTTG	TCATTGTCGG	CGCAACTATC	GGTATCAAGC	TGTTTAAGAA
	1501	ATTCACCTCG	AAAGCAAGCT	GATAAACCGA	TACAATTAAA	GGCTCCTTTT	GGAGCCTTTT
30	1561	TTTTTGGAGA	TTTTCAACGT	GAAAAAATTA	TTATTCGCAA	TTCCTTTAGT	TGTTCCTTTC
	1621	TATTCTCACA	GTGCACAGTC	TGTCGTGACG	CAGCCGCCCT	CAGTGTCTGG	GGCCCCAGGG
	1681	CAGAGGGTCA	CCATCTCCTG	CACTGGGAGC	AGCTCCAACA	TCGGGGCAGG	TTATGATGTA
	1741	CACTGGTACC	AGCAGCTTCC	AGGAACAGCC	CCCAAACTCC	TCATCTATGG	TAACAGCAAT
	1801	CGGCCCTCAG	GGGTCCCTGA	CCGATTCTCT	GGCTCCAAGT	CTGGCACCTC	AGCCTCCCTG
35	1861	GCCATCACTG	GGCTCCAGGC	TGAGGATGAG	GCTGATTATT	ACTGCCAGTC	CTATGACAGC
	1921	AGCCTGAGTG	GCCTTTATGT	CTTCGGAACT	GGGACCAAGG	TCACCGTCCT	AGGTCAGCCC
	1981	AAGGCCAACC	CCACTGTCAC	TCTGTTCCCG	CCCTCCTCTG	AGGAGCTCCA	AGCCAACAAG
	2041	GCCACACTAG	TGTGTCTGAT	CAGTGACTTC	TACCCGGGAG	CTGTGACAGT	GGCCTGGAAG

2101 GCAGATAGCA GCCCCGTCAA GGCGGGAGTG GAGACCACCA CACCCTCCAA ACAAAGCAAC

			AACAAGTACG					
			AGCTACAGCT					
			GAATGTTCAT					
			TAATGAAATA					
	5	2401	CCATGGCCGA	AGTTCAATTG	TTAGAGTCTG	GTGGCGGTCT	TGTTCAGCCT	GGTGGTTCTT
		2461	TACGTCTTTC	TTGCGCTGCT	TCCGGATTCA	CTTTCTCTTC	GTACGCTATG	TCTTGGGTTC
		2521	GCCAAGCTCC	TGGTAAAGGT	TTGGAGTGGG	TTTCTGCTAT	CTCTGGTTCT	GGTGGCAGTA
		2581	CTTACTATGC	TGACTCCGTT	AAAGGTCGCT	TCACTATCTC	TAGAGACAAC	TCTAAGAATA
			CTCTCTACTT					
	10	2701	AAGACTATGA	AGGTACTGGT	TATGCTTTCG	ACATATGGGG	TCAAGGTACT	ATGGTCACCG
		2761	TCTCTAGTGC	CTCCACCAAG	GGCCCATCGG	TCTTCCCCCT	GGCACCCTCC	TCCAAGAGCA
		2821	CCTCTGGGGG	CACAGCGGCC	CTGGGCTGCC	TGGTCAAGGA	CTACTTCCCC	GAACCGGTGA
		2881	CGGTGTCGTG	GAACTCAGGC	GCCCTGACCA	GCGGCGTCCA	CACCTTCCCG	GCTGTCCTAC
		2941	AGTCTAGCGG	ACTCTACTCC	CTCAGCAGCG	TAGTGACCGT	GCCCTCTTCT	AGCTTGGGCA
	15	3001	CCCAGACCTA	CATCTGCAAC	GTGAATCACA	AGCCCAGCAA	CACCAAGGTG	GACAAGAAAG
£ ==		3061	TTGAGCCCAA	ATCTTGTGCG	GCCGCTCATC	ACCACCATCA	TCACTCTGCT	GAACAAAAAC
m m		3121	TCATCTCAGA	AGAGGATCTG	AATGGTGCCG	CAGATATCAA	CGATGATCGT	ATGGCTGGCG
75		3181	CCGCTGAAAC	TGTTGAAAGT	TGTTTAGCAA	AACCCCATAC	AGAAAATTCA	TTTACTAACG
Ш		3241	TCTGGAAAGA	CGACAAAACT	TTAGATCGTT	ACGCTAACTA	TGAGGGTTGT	CTGTGGAATG
fi. ff.	20	3301	CTACAGGCGT	TGTAGTTTGT	ACTGGTGACG	AAACTCAGTG	TTACGGTACA	TGGGTTCCTA
ij		3361	TTGGGCTTGC	TATCCCTGAA	AATGAGGGTG	GTGGCTCTGA	GGGTGGCGGT	TCTGAGGGTG
Į.		3421	GCGGTTCTGA	GGGTGGCGGT	ACTAAACCTC	CTGAGTACGG	TGATACACCT	ATTCCGGGCT
£		3481	ATACTTATAT	CAACCCTCTC	GACGGCACTT	ATCCGCCTGG	TACTGAGCAA	AACCCCGCTA
		3541	ATCCTAATCC	TTCTCTTGAG	GAGTCTCAGC	CTCTTAATAC	TTTCATGTTT	CAGAATAATA
ļ.	25	3601	GGTTCCGAAA	TAGGCAGGGG	GCATTAACTG	TTTATACGGG	CACTGTTACT	CAAGGCACTG
1.1		3661	ACCCCGTTAA	AACTTATTAC	CAGTACACTC	CTGTATCATC	AAAAGCCATG	TATGACGCTT
I.		3721	ACTGGAACGG	TAAATTCAGA	GACTGCGCTT	TCCATTCTGG	CTTTAATGAA	GATCCATTCG
14		3781	TTTGTGAATA	TCAAGGCCAA	TCGTCTGACC	TGCCTCAACC	TCCTGTCAAT	GCTGGCGGCG
		3841	GCTCTGGTGG	TGGTTCTGGT	GGCGGCTCTG	AGGGTGGTGG	CTCTGAGGGT	GGCGGTTCTG
	30	3901	AGGGTGGCGG	CTCTGAGGGA	GGCGGTTCCG	GTGGTGGCTC	TGGTTCCGGT	GATTTTGATT
	•	3961	ATGAAAAGAT	GGCAAACGCT	AATAAGGGGG	CTATGACCGA	AAATGCCGAT	GAAAACGCGC
		4021	TACAGTCTGA	CGCTAAAGGC	AAACTTGATT	CTGTCGCTAC	TGATTACGGT	GCTGCTATCG
		4081	ATGGTTTCAT	TGGTGACGTT	TCCGGCCTTG	CTAATGGTAA	TGGTGCTACT	GGTGATTTTG
		4141	CTGGCTCTAA	TTCCCAAATG	GCTCAAGTCG	GTGACGGTGA	TAATTCACCT	TTAATGAATA
	35		ATTTCCGTCA					
			GCGCTGGTAA					
			TCTTTGCGTT					
			TACTGCGTAA					
			TTTCCTCGGT					
		1327	1110010001	1100110100	1.11.0111.011	COCCINICIO		1112222000

	4501	CTTCGGTAAG	ATAGCTATTG	CTATTTCATT	GTTTCTTGCT	CTTATTATTG	GGCTTAACTC
			*		CGCTCAATTA		
	4621	TGTTCAGTTA	ATTCTCCCGT	CTAATGCGCT	TCCCTGTTTT	TATGTTATTC	TCTCTGTAAA
	4681	GGCTGCTATT	TTCATTTTTG	ACGTTAAACA	AAAAATCGTT	TCTTATTTGG	ATTGGGATAA
5	4741	ATAATATGGC	TGTTTATTTT	GTAACTGGCA	AATTAGGCTC	TGGAAAGACG	CTCGTTAGCG
	4801	TTGGTAAGAT	TCAGGATAAA	ATTGTAGCTG	GGTGCAAAAT	AGCAACTAAT	CTTGATTTAA
	4861	GGCTTCAAAA	CCTCCCGCAA	GTCGGGAGGT	TCGCTAAAAC	GCCTCGCGTT	CTTAGAATAC
	4921	CGGATAAGCC	TTCTATATCT	GATTTGCTTG	CTATTGGGCG	CGGTAATGAT	TCCTACGATG
	4981	ААААТАААА	CGGCTTGCTT	GTTCTCGATG	AGTGCGGTAC	TTGGTTTAAT	ACCCGTTCTT
10	5041	GGAATGATAA	GGAAAGACAG	CCGATTATTG	ATTGGTTTCT	ACATGCTCGT	AAATTAGGAT
	5101	GGGATATTAT	TTTTCTTGTT	CAGGACTTAT	CTATTGTTGA	TAAACAGGCG	CGTTCTGCAT
	5161	TAGCTGAACA	TGTTGTTTAT	TGTCGTCGTC	TGGACAGAAT	TACTTTACCT	TTTGTCGGTA
	5221	CTTTATATTC	TCTTATTACT	GGCTCGAAAA	TGCCTCTGCC	TAAATTACAT	GTTGGCGTTG
	5281	TTAAATATGG	CGATTCTCAA	TTAAGCCCTA	CTGTTGAGCG	TTGGCTTTAT	ACTGGTAAGA
15	5341	ATTTGTATAA	CGCATATGAT	ACTAAACAGG	CTTTTTCTAG	TAATTATGAT	TCCGGTGTTT
	5401	ATTCTTATTT	AACGCCTTAT	TTATCACACG	GTCGGTATTT	CAAACCATTA	AATTTAGGTC
	5461	AGAAGATGAA	ATTAACTAAA	ATATATTTGA	AAAAGTTTTC	TCGCGTTCTT	TGTCTTGCGA
	5521	TTGGATTTGC	ATCAGCATTT	ACATATAGTT	ATATAACCCA	ACCTAAGCCG	GAGGTTAAAA
	5581	AGGTAGTCTC	TCAGACCTAT	GATTTTGATA	AATTCACTAT	TGACTCTTCT	CAGCGTCTTA
20	5641	ATCTAAGCTA	TCGCTATGTT	TTCAAGGATT	CTAAGGGAAA	ATTAATTAAT	AGCGACGATT
	5701	TACAGAAGCA	AGGTTATTCA	CTCACATATA	TTGATTTATG	TACTGTTTCC	ATTAAAAAAG
	5761	GTAATTCAAA	TGAAATTGTT	AAATGTAATT	AATTTTGTTT	TCTTGATGTT	TGTTTCATCA
	5821	TCTTCTTTTG	CTCAGGTAAT	TGAAATGAAT	AATTCGCCTC	TGCGCGATTT	TGTAACTTGG
	5881	TATTCAAAGC	AATCAGGCGA	ATCCGTTATT	GTTTCTCCCG	ATGTAAAAGG	TACTGTTACT
25	5941	GTATATTCAT	CTGACGTTAA	ACCTGAAAAT	CTACGCAATT	TCTTTATTTC	TGTTTTACGT
	6001	GCTAATAATT	TTGATATGGT	TGGTTCAATT	CCTTCCATAA	TTCAGAAGTA	TAATCCAAAC
	6061	AATCAGGATT	ATATTGATGA	ATTGCCATCA	TCTGATAATC	AGGAATATGA	TGATAATTCC
	6121	GCTCCTTCTG	GTGGTTTCTT	TGTTCCGCAA	AATGATAATG	TTACTCAAAC	TTTTAAAATT
	6181	AATAACGTTC	GGGCAAAGGA	TTTAATACGA	GTTGTCGAAT	TGTTTGTAAA	GTCTAATACT
<i>30</i>	6241	TCTAAATCCT	CAAATGTATT	ATCTATTGAC	GGCTCTAATC	TATTAGTTGT	TTCTGCACCT
	6301	AAAGATATTT	TAGATAACCT	TCCTCAATTC	CTTTCTACTG	TTGATTTGCC	AACTGACCAG
	6361	ATATTGATTG	AGGGTTTGAT	ATTTGAGGTT	CAGCAAGGTG	ATGCTTTAGA	TTTTTCATTT
	`6421	GCTGCTGGCT	CTCAGCGTGG	CACTGTTGCA	GGCGGTGTTA	ATACTGACCG	CCTCACCTCT
	6481	GTTTTATCTT	CTGCTGGTGG	TTCGTTCGGT	ATTTTTAATG	GCGATGTTTT	AGGGCTATCA
35	6541	GTTCGCGCAT	TAAAGACTAA	TAGCCATTCA	AAAATATTGT	CTGTGCCACG	TATTCTTACG
	6601	CTTTCAGGTC	AGAAGGGTTC	TATCTCTGTT	GGCCAGAATG	TCCCTTTTAT	TACTGGTCGT
	6661	GTGACTGGTG	AATCTGCCAA	TGTAAATAAT	CCATTTCAGA	CGATTGAGCG	TCAAAATGTA
	6721	GGTATTTCCA	TGAGCGTTTT	TCCTGTTGCA	ATGGCTGGCG	GTAATATTGT	TCTGGATATT
	6781	ACCAGCAAGG	CCGATAGTTT	GAGTTCTTCT	ACTCAGGCAA	GTGATGTTAT	TACTAATCAA
			- -			-	

The first seen for the first the circuit and circuit see given given given from the first first

							•
	6841	AGAAGTATTG	CTACAACGGT	TAATTTGCGT	GATGGACAGA	CTCTTTTACT	CGGTGGCCTC
	6901	ACTGATTATA	AAAACACTTC	TCAAGATTCT	GGCGTACCGT	TCCTGTCTAA	AATCCCTTTA
	6961	ATCGGCCTCC	TGTTTAGCTC	CCGCTCTGAT	TCCAACGAGG	AAAGCACGTT	ATACGTGCTC
	7021	GTCAAAGCAA	CCATAGTACG	CGCCCTGTAG	CGGCGCATTA	AGCGCGGCGG	GTGTGGTGGT
5	7081	TACGCGCAGC	GTGACCGCTA	CACTTGCCAG	CGCCCTAGCG	CCCGCTCCTT	TCGCTTTCTT
	7141	CCCTTCCTTT	CTCGCCACGT	TCGCCGGCTT	TCCCCGTCAA	GCTCTAAATC	GGGGGCTCCC
	7201	TTTAGGGTTC	CGATTTAGTG	CTTTACGGCA	CCTCGACCCC	AAAAAACTTG.	ĄTTTGGGTGA
	7261	TGGTTCACGT	AGTGGGCCAT	CGCCCTGATA	GACGGTTTTT	CGCCCTTTGA	CGTTGGAGTC
	7321	CACGTTCTTT	AATAGTGGAC	TCTTGTTCCA	AACTGGAACA	ACACTCAACC	CTATCTCGGG
10	7381	CTATTCTTTT	GATTTATAAG	GGATTTTGCC	GATTTCGGAA	CCACCATCAA	ACAGGATTTT
	7441	CGCCTGCTGG	GGCAAACCAG	CGTGGACCGC	TTGCTGCAAC	TCTCTCAGGG	CCAGGCGGTG
	7501	AAGGGCAATC	AGCTGTTGCC	CGTCTCACTG	GTGAAAAGAA	AAACCACCCT	GGATCCAAGC
	7561	TTGCAGGTGG	CACTTTTCGG	GGAAATGTGC	GCGGAACCCC	TATTTGTTTA	TTTTTCTAAA
	7621	TACATTCAAA	TATGTATCCG	CTCATGAGAC	AATAACCCTG	ATAAATGCTT	CAATAATATT
15	7681	GAAAAAGGAA	GAGTATGAGT	ATTCAACATT	TCCGTGTCGC	CCTTATTCCC	TTTTTTGCGG
	7741	CATTTTGCCT	TCCTGTTTTT	GCTCACCCAG	AAACGCTGGT	GAAAGTAAAA	GATGCTGAAG
	7801	ATCAGTTGGG	CGCACGAGTG	GGTTACATCG	AACTGGATCT	CAACAGCGGT	AAGATCCTTG
	7861	AGAGTTTTCG	CCCCGAAGAA	CGTTTTCCAA	TGATGAGCAC	TTTTAAAGTT	CTGCTATGTC
	7921	ATACACTATT	ATCCCGTATT	GACGCCGGGC	AAGAGCAACT	CGGTCGCCGG	GCGCGGTATT
20	7981	CTCAGAATGA	CTTGGTTGAG	TACTCACCAG	TCACAGAAAA	GCATCTTACG	GATGGCATGA
	8041	CAGTAAGAGA	ATTATGCAGT	GCTGCCATAA	CCATGAGTGA	TAACACTGCG	GCCAACTTAC
	8101	TTCTGACAAC	GATCGGAGGA	CCGAAGGAGC	TAACCGCTTT	TTTGCACAAC	ATGGGGGATC
	8161	ATGTAACTCG	CCTTGATCGT	TGGGAACCGG	AGCTGAATGA	AGCCATACCA	AACGACGAGC
	8221	GTGACACCAC	GATGCCTGTA	GCAATGCCAA	CAACGTTGCG	CAAACTATTA	ACTGGCGAAC
25	8281	TACTTACTCT	AGCTTCCCGG	CAACAATTAA	TAGACTGGAT	GGAGGCGGAT	AAAGTTGCAG
	8341	GACCACTTCT	GCGCTCGGCC	CTTCCGGCTG	GCTGGTTTAT	TGCTGATAAA	TCTGGAGCCG
	8401	GTGAGCGTGG	GTCTCGCGGT	ATCATTGCAG	CACTGGGGCC	AGATGGTAAG	CCCTCCCGTA
	8461	TCGTAGTTAT	CTACACGACG	GGGAGTCAGG	CAACTATGGA	TGAACGAAAT	AGACAGATCG
	8521	CTGAGATAGG	TGCCTCACTG	ATTAAGCATT	GGTAACTGTC	AGACCAAGTT	TACTCATATA
30	8581	TACTTTAGAT	TGATTTAAAA	CTTCATTTTT	AATTTAAAAG	GATCTAGGTG	AAGATCCTTT
	8641	TTGATAATCT	CATGACCAAA	ATCCCTTAAC	GTGAGTTTTC	GTTCCACTGT	ACGTAAGACC
	8701	CCCAAGCTTG	TCGACTGAAT	GGCGAATGGC	GCTTTGCCTG	GTTTCCGGCA	CCAGAAGCGG
	8761	TGCCGGAAAG	CTGGCTGGAG	TGCGATCTTC	CTGAGGCCGA	TACTGTCGTC	GTCCCCTCAA
	8821	ACTGGCAGAT	GCACGGTTAC	GATGCGCCCA	TCTACACCAA	CGTAACCTAT	CCCATTACGG
35	8881	TCAATCCGCC	GTTTGTTCCC	ACGGAGAATC	CGACGGGTTG	TTACTCGCTC	ACATTTAATG
	8941	TTGATGAAAG	CTGGCTACAG	GAAGGCCAGA	CGCGAATTAT	TTTTGATGGC	GTTCCTATTG
	9001	GTTAAAAAAT	GAGCTGATTT	AACAAAAATT	TAACGCGAAT	TTTAACAAAA	TATTAACGTT
	9061	TACAATTTAA	ATATTTGCTT	ATACAATCTT	CCTGTTTTTG	GGGCTTTTCT	GATTATCAAC
	9121	CGGGGTACAT	ATGATTGACA	TGCTAGTTTT	ACGATTACCG	TTCATCGATT	CTCTTGTTTG

9181 CTCCAGACTC TCAGGCAATG ACCTGATAGC CTTTGTAGAT CTCTCAAAAA TAGCTACCCT
9241 CTCCGGCATG AATTTATCAG CTAGAACGGT TGAATATCAT ATTGATGGTG ATTTGACTGT
9301 CTCCGGCCTT TCTCACCCTT TTGAATCTTT ACCTACACAT TACTCAGGCA TTGCATTTAA
9361 AATATATGAG GGTTCTAAAA ATTTTTATCC TTGCGTTGAA ATAAAGGCTT CTCCCGCAAA
9421 AGTATTACAG GGTCATAATG TTTTTGGTAC AACCGATTTA GCTTTATGCT CTGAGGCTTT
9481 ATTGCTTAAT TTTGCTAATT CTTTGCCTTG CCTGTATGAT TTATTGGATG TT

Table 200: Enzymes that either cut 15 or more human GLGs or have 5+-base recognition in FR3 Typical entry:

Typical entry: #sites REname Recognition GLGid#:base# GLGid#:base# GLGid#:base#..... 5 2 BstEII Ggtnacc 3 48: 3 1: There are 2 hits at base# 3 10 MaeIII gtnac 36 1: 2: 3: 4 4: 5: 6: 4 7: 8: 4 9: 10: 37: 4 4 4 11: 4 4 37: 58 38: 58 39: 39: 58 40: 38: 40: 58 41: 4 41: 58 42: 4 42: 58 43: 15 43: 58 44: 4 44: 58 45: 4 45: 58 46: 4 46: 58 47: 58 50: 58 47: 4 48: 49: There are 24 hits at base# 4 Tsp45I gtsac 33 20 2: 3: 4 4: 5: 6: 7: 4 8: 4 9: 4 10: 37: 4 4 11: 4 37: 58 38: 58 39: 58 38: 4 40: 40: 58 41: 58 42: 58 43: 43: 58 4 44: 44: 58 45: 4 45: 58 46: 4 46: 58 47: 58 47: 25 48: 4 49: 4 50: 58 There are 21 hits at base# HphI tcacc 45 1: 5 2: 5 3: 5 4: 5 5: 5 6: 5 30 7: 5 8: 5 11: 5 12: 13: 5 5 12: 11 14: 5 15: 5 16: 5 17: 5 18: 5 19: 5 20: 5 22: 23: 5 5 25: 21: 5 5 24: 5 26: 5 28: 29: 5 5 27: 5 5 30: 31: 5 32: 5 33: 5 34: 5 35: 5 5 37: 5 36: 35 38: 5 40: 43: 5 44: 5 45: 46: 5

There are 44 hits at base# 5

48:

49:

47:

	1:	9	1:	42	2:	42	3:	9	3:	42	4:	9	
	4:	42	5:	9	5:	42	6:	42	6:	78	7:	9	
	7:	42	8:	21	8:	42	9:	42	10:	42	11:	42	
5	12:	57	13:	48	13:	57	14:	57	31:	72	38:	9	
	48:	78	49:	78									
	The	re a	re 13	l hi	ts at	bas	se# 42						-
	The	re a	re :	l hi	ts at	bas	se# 48	Cou	ıld ca	ıse	ragge	iness.	
10	BsaJ:	[Cc	nngg										
	1:	14	2:	14	5:	14	6:	14			8:	14	
	8:	65	9:	14				14	12:	14	13:	14	
	14:	14							18:	65			
	20:	65						65	29:	65			
15	33:	65	34:	65				65	38:	65	39:	65	
	40:	65	42:	65	43:	65	48:	65	49:	65	50:	65	
	51:	14						•					1
	The	re a	re 23	3 hi	ts at	bas	se# 65						
	The	re a	re 14	4 hi	ts at	bas	se# 14						
20													
	AluI	AGc	t				4	12					
	1:	47	2:	47	3:	47	4:	47	5:	47	6:	47	
	7:	47	8:	4.7	9:	47	10:	47	11:	47	16:	63	
	23:	63	24:	63	25:	63	31:	63	32:	63	36:	63	
25	37.												
	<u>57.</u>	47	37:	<u>52</u>	<u> 38:</u>	47	38:	52		47	39:		
	40:						38: 41:		39:			52	
		47	40:	52	41:	47		52	39: 42:	47	42:	52 52	
	40:	47 47	40:	52 52	<u>41:</u> 44:	47 47	41:	52 52	39: 42: 45:	47 47	42:	52 52 52	
	40: 43: 46:	47 47 47	40: 43: 46:	52 52 52	41: 44: 47:	47 47 47	41: 44:	52 52 52	39: 42: 45:	47 47	42: 45:	52 52 52	
30	40: 43: 46: The	47 47 47 re a	40: 43: 46: re 23	52 52 52 52 6 hi	41: 44: 47: ts at	47 47 47 bas	41: 44: 47:	52 52 52	39: 42: 45: 49:	47 47 15	42: 45: 50:	52 52 52 47	
30	40: 43: 46: Then	47 47 47 re a	40: 43: 46: re 23 re 11	52 52 52 52 6 hi	41: 44: 47: ts at	47 47 47 bas	41: 44: 47: se# 47	52 52 52 52 Onl	39: 42: 45: 49:	47 47 15	42: 45: 50:	52 52 52 47	
30	40: 43: 46: The	47 47 47 re a	40: 43: 46: re 23 re 11	52 52 52 6 hi	41: 44: 47: .ts at	47 47 47 bas	41: 44: 47: se# 47	52 52 52 52 Onl	39: 42: 45: 49:	47 47 15	42: 45: 50: from	52 52 52 47 47	
30	40: 43: 46: The: The:	47 47 47 re a	40: 43: 46: re 23 re 11	52 52 52 6 hi	41: 44: 47: .ts at .ts at	47 47 47 bas bas	41: 44: 47: se# 47 se# 52	52 52 52 52 Onl	39: 42: 45: 49: y 5 ba	47 47 15	42: 45: 50: from	52 52 52 47 47	
<i>30</i>	40: 43: 46: Then Then BlpI 1:	47 47 47 re a	40: 43: 46: re 23 re 11	52 52 52 3 hi 1 hi	41: 44: 47: .ts at .ts at	47 47 47 bas bas	41: 44: 47: se# 47	52 52 52 52 Onl	39: 42: 45: 49: y 5 ba	47 47 15 ases	42: 45: 50: from 7:	52 52 52 47 47	
	10 15	4: 7: 5 12: 48: Therefore Therefore 10 BsaJJ 1: 8: 14: 20: 15 33: 40: 51: Therefore 20 AluI 1: 7: 23:	4: 42 7: 42 7: 42 5 12: 57 48: 78 There a There a 10 BsaJI Cc 1: 14 8: 65 14: 14 20: 65 15 33: 65 40: 65 51: 14 There a There a AluI AGC	4: 42 5: 7: 42 8: 5 12: 57 13: 48: 78 49: There are 1: There are 2: 8: 65 9: 14: 14 15: 20: 65 21: 15 33: 65 34: 40: 65 42: 51: 14 There are 2: There are 1: 20 AluI AGct 1: 47 2: 7: 47 8: 23: 63 24:	4: 42 5: 9 7: 42 8: 21 5 12: 57 13: 48 48: 78 49: 78 There are 11 hi There are 1 hi 10 BsaJI Conngg 1: 14 2: 14 8: 65 9: 14 14: 14 15: 65 20: 65 21: 65 15 33: 65 34: 65 40: 65 42: 65 51: 14 There are 23 hi There are 14 hi 20 AluI AGct 1: 47 2: 47 7: 47 8: 47	4: 42 5: 9 5: 7: 42 8: 21 8: 5 12: 57 13: 48 13: 48: 78 49: 78 There are 11 hits at There are 1 hits at 10 BsaJI Ccnngg 1: 14 2: 14 5: 8: 65 9: 14 10: 14: 14 15: 65 17: 20: 65 21: 65 22: 15 33: 65 34: 65 35: 40: 65 42: 65 43: 51: 14 There are 23 hits at There are 14 hits at 20 AluI AGct 1: 47 2: 47 3: 7: 47 8: 47 9:	4: 42 5: 9 5: 42 7: 42 8: 21 8: 42 5 12: 57 13: 48 13: 57 48: 78 49: 78 There are 11 hits at bas There are 1 hits at bas 10 BsaJI Conngg 1: 14 2: 14 5: 14 8: 65 9: 14 10: 14 14: 14 15: 65 17: 14 20: 65 21: 65 22: 65 40: 65 42: 65 43: 65 51: 14 There are 23 hits at bas There are 14 hits at bas There are 14 hits at bas 20 AluI AGct 1: 47 2: 47 3: 47 7: 47 8: 47 9: 47	4: 42 5: 9 5: 42 6: 7: 42 8: 21 8: 42 9: 5 12: 57 13: 48 13: 57 14: 48: 78 49: 78 There are 11 hits at base# 42 There are 1 hits at base# 48 10 BsaJI Ccnngg 1: 14 2: 14 5: 14 6: 8: 65 9: 14 10: 14 11: 14: 14 15: 65 17: 14 17: 20: 65 21: 65 22: 65 26: 15 33: 65 34: 65 35: 65 37: 40: 65 42: 65 43: 65 48: 51: 14 There are 23 hits at base# 65 There are 14 hits at base# 65 There are 14 hits at base# 14 20 AluI AGct 1: 47 2: 47 3: 47 4: 7: 47 8: 47 9: 47 10:	4: 42 5: 9 5: 42 6: 42 7: 42 8: 21 8: 42 9: 42 5 12: 57 13: 48 13: 57 14: 57 48: 78 49: 78 There are 11 hits at base# 42 There are 1 hits at base# 48 Cou 10 BsaJI Conngg 37 1: 14 2: 14 5: 14 6: 14 8: 65 9: 14 10: 14 11: 14 14: 14 15: 65 17: 14 17: 65 20: 65 21: 65 22: 65 26: 65 15 33: 65 34: 65 35: 65 37: 65 40: 65 42: 65 43: 65 48: 65 51: 14 There are 23 hits at base# 65 There are 14 hits at base# 14 20 AluI AGct 42 1: 47 2: 47 3: 47 4: 47 7: 47 8: 47 9: 47 10: 47	## 42 5: 9 5: 42 6: 42 6: 7: 42 8: 21 8: 42 9: 42 10: 5 12: 57 13: 48 13: 57 14: 57 31: 48: 78 49: 78 There are 11 hits at base# ## Could care ## 1	## 42 5: 9 5: 42 6: 42 6: 78 ## 7: 42 8: 21 8: 42 9: 42 10: 42 ## 5 12: 57 13: 48 13: 57 14: 57 31: 72 ## 48: 78 49: 78 ## There are 11 hits at base# 48 ## Could cause ## 75 14 5: 14 6: 14 7: 14 ## 8: 65 9: 14 10: 14 11: 14 12: 14 ## 14: 14 15: 65 17: 14 17: 65 18: 65 ## 20: 65 21: 65 22: 65 26: 65 29: 65 ## 15 33: 65 34: 65 35: 65 37: 65 38: 65 ## 40: 65 42: 65 43: 65 48: 65 49: 65 ## 51: 14 ## There are 23 hits at base# 65 ## There are 14 hits at base# 14 ## 20 ## Alul AGet 42 ## 1: 47 2: 47 3: 47 4: 47 5: 47 ## 7: 47 8: 47 9: 47 10: 47 11: 47 ## 11: 47 47 47 5: 47 ## 12: 47 47 47 5: 47 ## 13: 47 47 5: 47 ## 13: 47 47 5: 47 ## 13: 47 47 5: 47 ## 13: 47 47 5: 47 ## 13: 47 47 5: 47 ## 13: 47 47 5: 47 ## 13: 47 47 5: 47 ## 13: 47 47 5: 47 ## 13: 47 47 5: 47 ## 13: 47 47 5: 47 ## 13: 47 47 5: 47 ## 13: 47 47 47 5: 47 ## 13: 47 47 47 5: 47 ## 14: 47 47 5: 47 ## 15: 47 47 47 47 5: 47 ## 15: 47 47 47 47 5: 47 ## 15: 47 47 47 47 5: 47 ## 15: 47 47 47 47 5: 47 ## 15: 47 47 47 47 47 5: 47 ## 15: 47 47 47 47 47 47 ## 15: 47 47 47 47 47 ## 15: 47 47 47 47 ## 16: 78 ## 16: 78 78 ## 16: 78 78 ## 16: 78 78 ## 16: 78 78 ## 17: 47 47 47 47 ## 17: 47 47 47 ## 18: 47 47 ## 18: 47 47 ## 18: 47 47 ## 18: 47 47 ## 18: 47 47 ## 18: 47 47 ## 18: 47 47 ## 18: 47 ## 18: 48: 47 ## 18: 48: 47 ## 18: 48: 48: 48: 48: 48: 48: 48: 48: 48: 4	4: 42 5: 9 5: 42 6: 42 6: 78 7: 7: 42 8: 21 8: 42 9: 42 10: 42 11: 5 12: 57 13: 48 13: 57 14: 57 31: 72 38: 48: 78 49: 78 There are 11 hits at base# 42 There are 1 hits at base# 48 Could cause ragged 10 BsaJI Ccnngg 37 1: 14 2: 14 5: 14 6: 14 7: 14 8: 8: 65 9: 14 10: 14 11: 14 12: 14 13: 14: 14 15: 65 17: 14 17: 65 18: 65 19: 20: 65 21: 65 22: 65 26: 65 29: 65 30: 15 33: 65 34: 65 35: 65 37: 65 38: 65 39: 40: 65 42: 65 43: 65 48: 65 49: 65 There are 23 hits at base# 65 There are 14 hits at base# 14 20 AluI AGct 42 1: 47 2: 47 3: 47 4: 47 5: 47 6: 7: 47 8: 47 9: 47 10: 47 11: 47 16:	4: 42 5: 9 5: 42 6: 42 6: 78 7: 9 7: 42 8: 21 8: 42 9: 42 10: 42 11: 42 5 12: 57 13: 48 13: 57 14: 57 31: 72 38: 9 48: 78 49: 78 There are 11 hits at base# 42 There are 1 hits at base# 48 Could cause raggedness. 10 BsaJI Ccnngg 37 1: 14 2: 14 5: 14 6: 14 7: 14 8: 14 8: 65 9: 14 10: 14 11: 14 12: 14 13: 14 14: 14 15: 65 17: 14 17: 65 18: 65 19: 65 20: 65 21: 65 22: 65 26: 65 29: 65 30: 65 40: 65 42: 65 43: 65 37: 65 38: 65 39: 65 40: 65 42: 65 43: 65 48: 65 49: 65 50: 65 51: 14 There are 23 hits at base# 65 There are 14 hits at base# 14 20 AluI AGct 42 1: 47 2: 47 3: 47 4: 47 5: 47 6: 47 7: 47 8: 47 9: 47 10: 47 11: 47 16: 63 23: 63 24: 63 25: 63 31: 63 32: 63 36: 63

45: 48 46: 48 47: 48

There are 21 hits at base# 48

26

NlaIII CATG

```
1: 48
                     2: 28
                                        22: 36
                                                 23: 36
                                                           24: 36
                              19: 36
          25: 36
                              35: 36
                    26: 36
                                        37: 67
                                                 39: 67
                                                           40: 67
          41: 67
                    42: 67
                                                  45: 67
                                                           46: 67
                              43: 67
                                        44: 67
    5
          47: 67
          There are 10 hits at base# 67
                      7 hits at base# 36
          There are
         DdeI Ctnag
                                           71
   10
           1: 49
                     1: 58
                               2: 49
                                         2: 58
                                                  3: 49
                                                            3: 58
           3: 65
                     4: 49
                               4: 58
                                        5: 49
                                                  5: 58
                                                            5: 65
           6: 49
                     <u>6: 58</u>
                              6: 6<u>5</u>
                                        7: 49
                                                  7: 58
                                                            7: 65
           8: 49
                     8: 58
                                                  9: 65
                               9: 49
                                        9: 58
                                                           10: 49
                              11: 49
          10: 58
                   10: 65
                                        11: 58
                                                 11: 65
                                                           15: 58
   15
          16: 58
                    16: 65
                              17: 58
                                        18: 58
                                                 20: 58
                                                           21: 58
          22: 58
                    23: 58
                              23: 65
                                        24: 58
                                                 24: 65
                                                           <u>25: 58</u>
25: 65
                    26: 58
                              27: 58
                                       27: 65
                                                 28: 58
                                                           30: 58
1
Ü
          31: 58
                   31: 65
                              32: 58
                                       32: 65
                                                 35: 58
                                                           36: 58
         36: 65
                    37: 49
                              38: 49
                                        39: 26
                                                 39: 49
                                                           40: 49
   20
          41: 49
                              42: 49
THE COLUMN
                    42: 26
                                        43: 49
                                                 44: 49
                                                           45: 49
                    47: 49
          46: 49
                              48: 12
                                        49: 12
                                                 51: 65
          There are 29 hits at base# 58
He Hart II wille
          There are 22 hits at base# 49 Only nine base from 58
          There are 16 hits at base# 65 Only seven bases from 58
   25
4.1
         BglII Agatct
                                           11
           1: 61
                    2: 61
                               3: 61
                                         4: 61
                                                  5: 61
                                                            6: 61
                                                 51: 47
           7: 61
                     9: 61
                              10: 61
                                        11: 61
          There are 10 hits at base# 61
   30
         BstYI Rgatcy
                                           12
           1: 61
                     2: 61
                               3: 61
                                        4: 61
                                                  5: 61
                                                            6: 61
           7: 61
                     8: 61
                               9: 61
                                        10: 61
                                                 11: 61
                                                           51: 47
          There are 11 hits at base# 61
```

MwoI GCNNNNnngc

35

```
1: 64
                    2: 64
                                      4: 64
                             3: 64
                                                5: 64
                                                         6: 64
          7: 64
                    8: 64
                             9: 64
                                     10: 64
                                               11: 64
                                                        16: 57
         20: 57
                  27: 57
                            35: 57
                                      48: 67
                                               49: 67
    5
         There are 11 hits at base# 64
         There are
                    4 hits at base# 57
         There are
                    2 hits at base# 67 Could be ragged.
        MslI CAYNNnnRTG
                                         44
   10
                                      4: 72
          1: 72
                   2: 72
                                                5: 72
                             3: 72
                                                         6: 72
          7: 72
                   8: 72
                             9: 72
                                     10: 72
                                               11: 72
                                                        15: 72
         17: 72
                  18: 72
                            19: 72
                                     21: 72
                                               23: 72
                                                        24: 72
         25: 72
                  26: 72
                            28: 72
                                     29: 72
                                               30: 72
                                                        31: 72
         32: 72
                  33: 72
                            34: 72
                                     35: 72
                                               36: 72
                                                        37: 72
   15
         38: 72
                  39: 72
                            40: 72
                                     41: 72
                                               42: 72
                                                        43: 72
44: 72
                  45: 72
                            46: 72
                                     47: 72
                                                        49: 72
                                               48: 72
         50: 72
                  51: 72
There are 44 hits at base# 72
20
        BsiEI CGRYcg
                                        23
1: 74
                   3: 74
                             4: 74
                                      5: 74
                                                7: 74
                                                         8: 74
          9: 74
                                     17: 74
                                               22: 74
17 8 8 4.
                  10: 74
                            11: 74
                                                        30: 74
         33: 74
                  34: 74
                            37: 74
                                     38: 74
                                               39: 74
                                                        40: 74
         41: 74
                  42: 74
                            45: 74
                                     46: 74
                                               47: 74
         There are 23 hits at base# 74
        Eael Yggccr
                                        23
                                      5: 74
          1: 74
                   3: 74
                             4: 74
                                                7: 74
                                                         8: 74
          9: 74
                  10: 74
                            11: 74
                                     17: 74
                                               22: 74
                                                        30: 74
   30
         33: 74
                  34: 74
                            37: 74
                                     38: 74
                                               39: 74
                                                        40: 74
         41: 74
                  42: 74
                            45: 74
                                     46: 74
                                               47: 74
         There are 23 hits at base# 74
        EagI Cggccg
                                        23
   35
          1: 74
                   3: 74
                             4: 74
                                      5: 74
                                                7: 74
                                                         8: 74
          9: 74
                  10: 74
                            11: 74
                                     17: 74
                                               22: 74
                                                        30: 74
```

Hpy188I TCNga

=

```
5
         HaeIII GGcc
                                          27
                                                 7: 75
                                                           8: 75
           1: 75
                    3: 75
                              4: 75
                                        5: 75
           9: 75
                   10: 75
                             11: 75
                                       16: 75
                                                17: 75
                                                          20: 75
          22: 75
                   30: 75
                             33: 75
                                       34: 75
                                                37: 75
                                                          38: 75
                   40: 75
          39: 75
                                       42: 75
                                                45: 75
                                                          46: 75
                             41: 75
   10
                   48: 63
                             49: 63
          47: 75
          There are 25 hits at base# 75
        Bst4CI ACNgt 65°C
                                  63 Sites There is a third isoschismer
           1: 86
                    2: 86
                              3: 86
                                        4: 86
                                                 5: 86
                                                           6: 86
   15
           7: 34
                    7: 86
                              8: 86
                                       9: 86
                                                10: 86
                                                          11: 86
IJ
          12: 86
                   13: 86
                             14: 86
                                       15: 36
                                                15: 86
                                                          16: 53
J
          16: 86
                   17: 36
                             17: 86
                                       18: 86
                                                19: 86
                                                          20: 53
10
                   21: 36
                             21: 86
                                       22: 0
                                                22: 86
                                                          23: 86
          20: 86
The Control
                                                27: 86
          24: 86
                   25: 86
                             26: 86
                                       27: 53
                                                          28: 36
   20
          28: 86
                   29: 86
                             30: 86
                                       31: 86
                                                32: 86
                                                          33: 36
(11)
          33: 86
                   34: 86
                             35: 53
                                       35: 86
                                                36: 86
                                                          37: 86
m 4...... H
          38: 86
                   39: 86
                             40: 86
                                       41: 86
                                                42: 86
                                                          43: 86
          44: 86
                             46: 86
                                                          49: 86
                   45: 86
                                       47: 86
                                                48: 86
14
          50: 86
                   51: 0
                             51: 86
25
          There are 51 hits at base# 86 All the other sites are well away
         HpyCH4III ACNgt
                                        63
                              3: 86
           1: 86
                    2: 86
                                        4: 86
                                               5: 86
                                                           6: 86
           7: 34
                    7: 86
                              8: 86
                                        9: 86
                                                10: 86
                                                          11: 86
   30
          12: 86
                   13: 86
                             14: 86
                                       15: 36
                                                15: 86
                                                          16: 53
          16: 86
                   17: 36
                             17: 86
                                       18: 86
                                                19: 86
                                                          20: 53
          20: 86
                   21: 36
                             21: 86
                                       22: 0
                                                22: 86
                                                          23: 86
          24: 86
                   25: 86
                             26: 86
                                       27: 53
                                                27: 86
                                                          28: 36
          28: 86
                   29: 86
                             30: 86
                                       31: 86
                                                32: 86
                                                          33: 36
```

33: 74

41: 74

35

33: 86

38: 86

34: 86

39: 86

35: 53

40: 86

35: 86

41: 86

34: 74

42: 74

There are 23 hits at base# 74

37: 74

45: 74

38: 74

46: 74

39: 74

47: 74

11.00

36: 86

42: 86

37: 86

43: 86

40: 74

There are 51 hits at base# 86 5 HinfI Ganto 43 2: 2 3: 2 4: 2 5: 2 7: 2 6: 2 8: 2 9: 2 9: 22 10: 2 11: 2 15: 2 16: 17: 2 18: 2 19: 2 19: 22 20: 21: 2 23: 2 24: 2 25: 2 26: 2 27: 10 28: 2 29: 2 30: 2 31: 2 32: 2 33: 2 33: 22 34: 22 35: 2 36: 2 37: 2 38: 2 40: 2 43: 2 44: 2 45: 2 46: 2 47: 2 50: 60 There are 38 hits at base# 2 15 1.7 th MlyI GAGTCNNNNn 18 O 3: 2 2: 2 4: 2 5: 2 6: 2 7: 2 Ų 8: 2 9: 2 10: 2 11: 2 37: 2 38: 2 7. 40: 2 43: 2 44: 2 45: 2 46: 2 47: 2 IJ 20 There are 18 hits at base# 2 [ħ s PleI gagtc 18 2: 2 3: 2 4: 2 5: 2 6: 2 7: 2 8: 2 9: 2 10: 2 11: 2 37: 2 38: 2 25 40: 2 43: 2 44: 2 45: 2 46: 2 47: 2 There are 18 hits at base# 2 Acil Ccqc 24 2: 26 9: 14 10: 14 11: 14 27: 74 37: 62 37: 65 38: 62 39: 65 40: 62 40: 65 41: 65 30 43: 65 42: 65 43: 62 44: 62 44: 65 45: 62 <u>47: 62</u> 46: 62 <u>47: 65</u> 48: 35 48: 74 49: 74 There are 8 hits at base# 62 There are 8 hits at base# 65 There are 3 hits at base# 14 35 There are 3 hits at base# 74 There are 1 hits at base# 26

45: 86 46: 86

51: 86

51: 0

47: 86 48: 86 49: 86

44: 86

50: 86

.

1 hits at base# 35

There are

```
11: 16
          8: 91
                  9: 16
                           10: 16
                                             37: 67
                                                      39: 67
         40: 67
                  42: 67
                                    45: 67
                           43: 67
                                             46: 67
         There are 7 hits at base# 67
    5
         There are 3 hits at base# 16
         There are 1 hits at base# 91
        BsiHKAI GWGCWc
                                       20
          2: 30
                  4: 30
                          6: 30
                                    7: 30
                                            9: 30
                                                      10: 30
   10
         12: 89
                13: 89
                           14: 89
                                    37: 51
                                             38: 51
                                                      39: 51
         40: 51
                  41: 51
                           42: 51
                                    43: 51
                                             44: 51
                                                      45: 51
         46: 51
                  47: 51
         There are 11 hits at base# 51
   15
        Bsp1286I GDGCHc
                                       20
          2: 30
                 4: 30
                           6: 30
                                     7: 30
                                            9: 30
1. C.
                                                      10: 30
         12: 89
                 13: 89
                           14: 89
                                    37: 51
                                             38: 51
                                                      39: 51
O
         40: 51
                  41: 51
                           42: 51
                                    43: 51
                                             44: 51
                                                      45: 51
IJ
                  47: 51
         46: 51
١.]
LJ 20
         There are 11 hits at base# 51
IJ
[]
        HgiAI GWGCWc
                                       20
₽
= 2
          2: 30
                 4: 30
                            6: 30
                                     7: 30
                                            9: 30
                                                      10: 30
         12: 89
                13: 89
                           14: 89
                                    37: 51
                                             38: 51
                                                      39: 51
  25
         40: 51
                  41: 51
                           42: 51
                                    43: 51
                                             44: 51
                                                      45: 51
F. I
                  47: 51
         46: 51
There are 11 hits at base# 51
        BsoFI GCngc
                                       26
                                    6: 53
   30
          2: 53
                 3: 53
                            5: 53
                                            7: 53
                                                       8: 53
          8: 91
                  9: 53
                           10: 53
                                    11: 53
                                             31: 53
                                                      36: 36
         37: 64
                 39: 64
                           40: 64
                                    41: 64
                                             42: 64
                                                      43: 64
         44: 64
                  45: 64
                           46: 64
                                    47: 64
                                             48: 53
                                                      49: 53
         50: 45
                  51: 53
   35
         There are 13 hits at base# 53
         There are 10 hits at base# 64
        TseI Gcwgc
                                       17
          2: 53
                3: 53 5: 53 6: 53
                                           7: 53
                                                       8: 53
```

-"- Gcgg

..

```
46: 64
                                                     48: 53
                                                                          49: 53
                                                                                                           50: 45
                                                                                                                                     51: 53
                           There are 13 hits at base# 53
            5
                        MnlI gagg
                                                                                                                   34
                              3: 67
                                                        3: 95
                                                                                  4: 51
                                                                                                           5: 16
                                                                                                                                  5: 67
                                                                                                                                                            6: 67
                             7: 67
                                                  8: 67
                                                                                   9: 67
                                                                                                           10: 67
                                                                                                                                     11: 67
                                                                                                                                                                15: 67
                           16: 67
                                                  17: 67
                                                                                19: 67
                                                                                                           20: 67
                                                                                                                                     21: 67
                                                                                                                                                                22: 67
                           23: 67
                                                   24: 67
                                                                                25: 67
                                                                                                           26: 67
                                                                                                                                     27: 67
                                                                                                                                                                28: 67
         10
                          29: 67
                                                  30: 67
                                                                                31: 67
                                                                                                           32: 67
                                                                                                                                     33: 67
                                                                                                                                                                34: 67
                           35: 67
                                                     36: 67
                                                                                50: 67
                                                                                                           51: 67
                           There are 31 hits at base# 67
                        HpyCH4V TGca
                                                                                                                   34
         15
                              5: 90
                                                  6: 90
                                                                                                           12: 90
                                                                                11: 90
                                                                                                                                     13: 90
                                                                                                                                                                14: 90
The start stare of the start of the start 
                           15: 44
                                                  16: 44
                                                                                16: 90
                                                                                                           17: 44
                                                                                                                                   18: 90
                                                                                                                                                               19: 44
                          20: 44
                                                  21: 44
                                                                                22: 44
                                                                                                          23: 44
                                                                                                                                    24: 44
                                                                                                                                                               25: 44
                           26: 44
                                                  27: 44
                                                                                27: 90
                                                                                                           28: 44
                                                                                                                                     29: 44
                                                                                                                                                                33: 44
                           34: 44
                                                    35: 44
                                                                                35: 90
                                                                                                           36: 38
                                                                                                                                     48: 44
                                                                                                                                                                49: 44
         20
                           50: 44
                                                     50: 90
                                                                                51: 44
                                                                                                           51: 52
                           There are 21 hits at base# 44
                           There are 1 hits at base# 52
                        AccI GTmkac
                                                                                                                   13 5-base recognition
[]. 25
                             7: 37
                                                     11: 24
                                                                                37: 16
                                                                                                           38: 16
                                                                                                                                     39: 16
                                                                                                                                                                40: 16
                           41: 16
                                                     42: 16
                                                                                43: 16
                                                                                                           44: 16
                                                                                                                                     45: 16
                           47: 16
                           There are 11 hits at base# 16
         30
                        SacII CCGCgg
                                                                                                                      8
                                                                                                                                  6-base recognition
                             9: 14
                                                     10: 14
                                                                               11: 14
                                                                                                          37: 65
                                                                                                                                     39: 65
                                                                                                                                                               40: 65
                                                     43: 65
                           42: 65
                           There are 5 hits at base# 65
                          There are 3 hits at base# 14
         35
                        TfiI Gawtc
                                                                                                                   24
                              9: 22
                                                                                                           17: 2
                                                    15: 2
                                                                                16: 2
                                                                                                                                     18: 2
                                                                                                                                                               19: 2
                          19: 22
                                                    20: 2
                                                                                21: 2
                                                                                                          23: 2
                                                                                                                                     24: 2
                                                                                                                                                               25: 2
```

10: 53 11: 53

31: 53

36: 36

45: 64

9: 53

8

ļ÷

٦.』

ļi

		26:	2	27:	2	28:	2	29:	2	30:	2	31:	2
		32:	2	33:	2	33:	22	34:	22	35:	2	36:	2
		The	re ar	e 20	hit	s at	bas	e# 2					
	5	BsmA.	I Nnn	nnnga	agac				19				
		15:	11	16:	11	20:	11	21:	11	22:	11	23:	11
		24:	11	25:	11	26:	11	27:	11	28:	11	28:	56
		30:	11	31:	11	32:	11	35:	11	36:	11	44:	87
		48:	87						•				
	10	The	re ar	e 16	hit	s at	bas	e# 11					
		_	ctcc	-					19				
		15:		16:		17:		18:		20:		21:	12
	1.5	22:		23:			12	25:		26:		27:	
	15	28:		30:	12	31:	12	32:	12	34:	12	35:	12
[]		36:	12										
		The	re ar	e 19	hit	s at	bas	e# 12					
The first compact.											•		
1,11	20	XmnI							12				
	20	37:		38:		39:		40:		41:		42:	
n		43:		44:		45:		46:	30	47:	30	50:	30
=	•	Ther	re are	e 12	hit	s at	bas	e# 30					
# # # # # # # # # # # # # # # # # # #													
la La	25	BsrI		_					12				
dull dan De	25	37:			32			40:		41:		42:	
7		43:		44:		45:		46:	32	47:	32	50:	32
14		Ther	ce are	e 12	hit	s at	bas	e# 32					
		BanII	CPC	~V~				-	l1				
	30				51	30.	51	40:		41.	E 1	42.	E 1
								46:				42.	31
								e# 51		7/.	JI		
						o ac	Zus	C# 01					
		Ecl13	86I G <i>A</i>	AGctc	:			1	11				
	35					39:	51	40:	_	41:	51	42:	51
								46:					
								e# 51					

SacI GAGCTc

37: 51 38: 51 39: 51 40: 51 41: 51 42: 51

43: 51 44: 51 45: 51 46: 51 47: 51

There are 11 hits at base# 51

5

Table 206: Synthetic 3-23 FR3 of human heavy chains showning positions of possible cleavage sites

```
! Sites engineered into the synthetic gene are shown in upper case DNA
        ! with the RE name between vertical bars (as in | XbaI |).
        ! RERSs frequently found in GLGs are shown below the synthetic sequence
        ! with the name to the right (as in gtn ac=MaeIII(24), indicating that
        ! 24 of the 51 GLGs contain the site).
                                                                |---FR3---
   10
                                                                 89 90 (codon # in
                                                                 R F synthetic 3-23)
                                                                |cgc|ttc| 6
           Allowed DNA
                                                                |cgn|tty|
                                                                agr
   15
                                                                  ga ntc = HinfI(38)
                                                                  ga gtc = PleI(18)
                                                                  ga wtc = TfiI(20)
                                                                     gtn ac = MaeIII(24)
                                                                     gts ac = Tsp45I(21)
   20
                                                                      tc acc = HphI(44)
                -----FR3-----
                 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
                 TISRDNSKNTLYLQM
   25
               |act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|
allowed|acn|ath|tcn|cgn|gay|aay|tcn|aar|aay|acn|ttr|tay|ttr|car|atg!!
                       |agy|agr|
                                     lagyl
                                                    |ctn| |ctn|
                           ga|gac = BsmAI(16)
                                                                  ag ct = AluI(23)
ľŌ
                      c|tcc ag = BpmI(19)
                                                                   g ctn agc = BlpI(21)
U
   30
                                            g aan nnn ttc = XmnI(12)
٦...
                       | XbaI
                                                              tg ca = HpyCH4V(21)
Ų
               ---FR3-----
                106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
   35
                   S L R A E D T A V Y Y C A K
               |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgc|gct|aaa|
2
        !allowed|aay|tcn|ttr|cgn|gcn|gar|gay|acn|gcn|gtn|tay|tay|tgy|gcn|aar|
                   |agy|ctn|agr|
                            -
                               cc nng g = BsaJI(23)
                                                          ac ngt = Bst4CI(51)
   40
                           aga tct = BglII(10)
                                                          ac ngt = HpyCH4III (51)
                           Rga tcY = BstYI(11)
                                                 1
                                                          ac ngt = TaaI(51)
                                         c ayn nnn rtc = MslI(44)
                                           cg ryc g = BsiEI(23)
                                           yg gcc r = EaeI(23)
   45
                                           cg gcc g = EagI(23)
                                           |g gcc = HaeIII(25)
                                   gag g = MnlI(31)
                      |AflII |
                                           | PstI |
```

Table 217: Human HC GLG FR1 Sequences

VH Exon - Nucleotide sequence alignment

		AII PYC	111	MUCI	COLI	ue s	eque	nce	arry	muen											
		VH1							•												
		1-02	CAG	GTG	CAG	CTG	GTG	CAG	тст	GGG	GCT	GAG	GTG	AAG	AAG	ССТ	GGG	GCC	TCA	GTG	AAG
	5		GTC	TCC	TGC	AAG	GCT	тст	GGA	TAC	ACC	TTC	ACC								
	•	1-03			cag									aaσ	aaσ	cct	aaa	acc	tca	ata	aad
		1 00			tgc									uug	uug	-	999	goo	ccu	9-9	uug
		1-08	-		cag	_	-							224	220	cct	aaa	acc	+~=	ata	224
		1 00			tgc									aug	aag	CCL	àaa	gcc	cca	gcg	aay
	10	1-18	_		cag	_	-							224	224	cat	~~~	~ ~~	+00	~+~	225
	10	1 10			tgc									aay	aay	CCC	999	gcc	cca	gtg	aay
		1-24	_		cag	_	-							224	224	cct	~~~	~~~	+	~+~	224
		1 21			tgc									aay	aay	CCC	999	gcc	cca	grg	aay
		1-45	_		cag	_	-							224		7.0+		maa.	+		
	15	1 43												aay	aay	ACL	ggg	100	tta	grg	aay
	15	1-46	-		tgc	_	-									+					
		1-40			cag	_		_			-			aay	aay	CCL	ggg	gcc	ica	grg	aay
		1-58			tgc		-											3			
		1-30			cag									aag	aag	CCL	999	ACC	tca	gtg	aag
	20	1 60	-		tgc	_	-											m			
j	20	1-69			cag	-	-	_			_			aag	aag	CCT	ggg	TCC	tcG	gtg	aag
7		1 -	_		tgc	-	-											-			
		1-e	_		cag	-		_			•			aag	aag	CCT	ggg	TCC	tcG	gtg	aag
1,01					tgc	_	=											_	_		_
ħ	25	1-f			-	_	_	_			-			aag	aag	cct	ggg	gcT	Aca	gtg	aaA
25,	23		Atc	tcc	tgc	aag	gīt	tct	gga	tac	acc	ttc	acc								
***************************************		VH2																			
÷	•	2-05			ACC									GTG	AAA	CCC	ACA	CAG	ACC	CTC	ACG
					TGC																
F 154 di.	20	2-26			acc									gtg	aaa	ccc	aca	Gag	acc	ctc	acg
å	30		-		tgc								_								_
		2-70			acc									gtg	aaa	ccc	aca	cag	acc	ctc	acA
			ctg	acc	tgc	acc	ttc	tct	ggg	ttc	tca	ctc	agc								
		VH3																			
	25	3-07			CAG									GTC	CAG	CCT	GGG	GGG	TCC	CTG	AGA
	35				TGT																
		3-09			cag									gtA	cag	cct	ggC	Agg	tcc	ctg	aga
					tgt	_	-														
		3-11			cag									gtc	Aag	cct	ggA	ggg	tcc	ctg	aga
	40				tgt																
	40	3-13			cag	_							•	gtA	cag	cct	ggg	ggg	tcc	ctg	aga
		_			tgt	-	_						-								
		3-15			cag									gtA	Aag	cct	ggg	ggg	tcc	ctT	aga
			ctc	tcc	tgt	gca	gcc	tct	gga	ttc	acT	ttC	agt								
		3-20	gag	gtg	cag	ctg	gtg	gag	tct	ggg	gga	ggT	Gtg	gtA	cGg	cct	ggg	ggg	tcc	ctg	aga

3-21 gag gtg cag ctg gtg gag tct ggg gga ggc Ctg gtc Aag cct ggg ggg tcc ctg aga ctc tcc tgt gca gcc tct gga ttc acc ttC agt 3-23 gag gtg cag ctg Ttg gag tct ggg gga ggc ttg gtA cag cct ggg ggg tcc ctg aga 5 ctc tcc tgt gca gcc tct gga ttc acc ttt agC 3-30 Cag gtg cag ctg gtg gag tct ggg gga ggc Gtg gtc cag cct ggg Agg tcc ctg aga ctc tcc tgt gca gcc tct gga ttc acc ttC agt 3-30.3 Cag gtg cag ctg gtg gag tct ggg gga ggc Gtg gtc cag cct ggg Agg tcc ctg aga ctc tcc tgt gca gcc tct gga ttc acc ttC agt 10 3-30.5 Cag gtg cag ctg gtg gag tct ggg gga ggc Gtg gtc cag cct ggg Agg tcc ctg aga ctc tcc tgt gca gcc tct gga ttc acc ttC agt Cag gtg cag ctg gtg gag tct ggg gga ggc Gtg gtc cag cct ggg Agg tcc ctg aga ctc tcc tgt gca gcG tct gga ttc acc ttC agt 3-43 gaA gtg cag ctg gtg gag tct ggg gga gTc Gtg gtA cag cct ggg ggg tcc ctg aga 15 ctc tcc tgt gca gcc tct gga ttc acc ttt GAt 3-48 gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtA cag cct gqg ggg tcc ctg aga ctc tcc tgt gca gcc tct gga ttc acc ttC agt 3-49 gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtA cag ccA ggg Cgg tcc ctg aga that the die the trail that that ctc tcc tgt Aca gcT tct gga ttc acc ttt Ggt 20 3-53 gag gtg cag ctg gtg gag Act ggA gga ggc ttg Atc cag cct ggg ggg tcc ctg aga ctc tcc tgt gca gcc tct ggG ttc acc GtC agt 3-64 gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc cag cct ggg ggg tcc ctg aga ctc tcc tgt gca gcc tct gga ttc acc ttC agt 3-66 gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc cag cct ggg ggg tcc ctg aga (n 25 ctc tcc tgt gca gcc tct gga ttc acc GtC agt 1 3-72 gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc cag cct ggA ggg tcc ctg aga ctc tcc tgt gca gcc tct gga ttc acc ttC agt ļ 3-73 gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc cag cct ggg ggg tcc ctg aAa 4.4 ctc tcc tgt gca gcc tct ggG ttc acc ttC agt *30* 3-74 gag gtg cag ctg gtg gag tcC ggg gga ggc ttA gtT cag cct ggg ggg tcc ctg aga ctc tcc tgt gca gcc tct gga ttc acc ttC agt 3-d gag gtg cag ctg gtg gag tct Cgg gga gTc ttg gtA cag cct ggg ggg tcc ctg aga ctc tcc tgt gca gcc tct gga ttc acc GtC agt VH4 35 4-04 CAG GTG CAG CTG CAG GAG TCG GGC CCA GGA CTG GTG AAG CCT TCG GGG ACC CTG TCC CTC ACC TGC GCT GTC TCT GGT GGC TCC ATC AGC cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcg gAC acc ctg tcc 4-28 ctc acc tgc gct gtc tct ggt TAc tcc atc agc 4-30.1 cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcA CAg acc ctg tcc 40 ctc acc tgc Act gtc tct ggt ggc tcc atc agc 4-30.2 cag Ctg cag ctg cag gag tcC ggc Tca gga ctg gtg aag cct tcA CAg acc ctg tcc ctc acc tgc gct gtc tct ggt ggc tcc atc agc 4-30.4 cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcA CAg acc ctg tcc ctc acc tgc Act gtc tct ggt ggc tcc atc agc

ctc tcc tgt gca gcc tct gga ttc acc ttt GAt

	4-31	cag	gtg	cag	ctg	cag	gag	tcg	ggc	cca	gga	ctg	gtg	aag	cct	tcA	CAg	acc	ctg	tcc
		ctc	acc	tgc	Act	gtc	tct	ggt	ggc	tcc	atc	agc								
	4-34	cag	gtg	cag	ctA	cag	Cag	tGg	ggc	Gca	gga	ctg	Ttg	aag	cct	tcg	gAg	acc	ctg	tcc
		ctc	acc	tgc	gct	gtc	tAt	ggt	ggG	tcc	Ttc	agT								
5	4-39	cag	Ctg	cag	ctg	cag	gag	tcg	ggc	сса	gga	ctg	gtg	aag	cct	tcg	gAg	acc	ctg	tcc
		ctc	acc	tgc	Act	gtc	tct	ggt	ggc	tcc	atc	agc								
	4-59	cag	gtg	cag	ctg	cag	gag	tcg	ggc	cca	gga	ctg	gtg	aag	cct	tcg	gAg	acc	ctg	tcc
		ctc	acc	tgc	Act	gtc	tct	ggt	ggc	tcc	atc	agT						-		
	4-61	cag	gtg	cag	ctg	cag	gag	tcg	ggc	cca	gga	ctg	gtg	aag	cct	tcg	gAg	acc	ctg	tcc
10		ctc	acc	tgc	Act	gtc	tct	ggt	ggc	tcc	Gtc	agc								
	4-b	cag	gtg	cag	ctg	cag	gag	tcg	ggc	cca	gga	ctg	gtg	aag	cct	tcg	gAg	acc	ctg	tcc
		ctc	acc	tgc	gct	gtc	tct	ggt	TAc	tcc	atc	agc								
	VH5																			
	5-51	GAG	GTG	CAG	CTG	GTG	CAG	TCT	GGA	GCA	GAG	GTG	AAA	AAG	CCC	GGG	GAG	TCT	CTG	AAG
15		ATC	TCC	TGT	AAG	GGT	TCT	GGA	TAC	AGC	TTT	ACC								
	5-a	gaA	gtg	cag	ctg	gtg	cag	tct	gga	gca	gag	gtg	aaa	aag	ccc	ggg	gag	tct	ctg	aGg
		atc	tcc	tgt	aag	ggt	tct	gga	tac	agc	ttt	acc								
	VH6																			
	6-1	CAG	GTA	CAG	CTG	CAG	CAG	TCA	GGT	CCA	GGA	CTG	GTG	AAG	ccc	TCG	CAG	ACC	CTC	TCA
<i>20</i>		CTC	ACC	TGT	GCC	ATC	TCC	GGG	GAC	AGT	GTC	TCT								
	VH7																			
	7-4.1	CAG	GTG	CAG	CTG	GTG	CAA	TCT	GGG	TCT	GAG	TTG	AAG	AAG	CCT	GGG	GCC	TCA	GTG	AAG
		GTT	TCC	TGC	AAG	GCT	TCT	GGA	TAC	ACC	TTC	ACT								

BsgI GTGCAG 71 (cuts 16/14 bases to right) 1: 4 1: 13 2: 13 3: 4 3: 13 4: 13 6: 13 7: 9: 4 7: 13 8: 13 4 9: 13 5 10: 10: 13 15: 15: 65 4 16: 65 4 16: 17: 17: 65 18: 4 18: 65 19: 19: 65 4 4 20: 20: 65 21: 65 22: 65 21: 22: 23: 23: 65 24: 24: 65 25: 4 25: 65 26: 26: 65 27: 27: 65 28: 28: 65 4 4 4 10 29: 4 30: 4 30: 65 31: 4 31: 65 32: 4 32: 65 33: 4 33: 65 34: 4 34: 65 35: 4 35: 65 36: 4 36: 65 37: 39: 4 38: 4 41: 4 42: 4 43: 4 45: 46: 4 47: 4 4 48: 4 48: 13 49: 4 49: 13 51: 4 15 There are 39 hits at base# O There are 21 hits at base# 65 1 C -"- ctgcac 9 Ų 12: 63 13: 63 14: 63 39: 63 41: 63 42: 63 إيا LJ 20 44: 63 45: 63 46: 63 O BbvI GCAGC 65 ţħ 1: 6 3: 6 6: 6 7: 6 8: 6 9: 6 Ξ Ü 10: 6 15: 6 15: 67 16: 16: 67 17: 6 6 === 17: 67 18: 6 18: 67 19: 6 20: 19: 67 6 25 20: 67 21: 6 21: 67 22: 6 22: 67 23: 6 23: 67 24: 67 24: 6 25: 6 25: 67 26: [] 6 26: 67 27: 6 27: 67 28: 28: 67 29: 6 6 30: 6 30: 67 31: 32: 67 6 31: 67 32: 6 33: 33: 67 34: 6 6 34: 67 35: 6 35: 67 30 36: 6 36: 67 37: 6 38: 6 39: 6 40: 6 41: 6 42: 6 43: 6 44: 6 45: 6 46: 6 47: 6 48: 6 49: 6 **50:** 12 51: 6 There are 43 hits at base# 6 Bolded sites very near sites listed below 35 There are 21 hits at base# 67 gctgc 13 37: 9 38: 40: 9 39: 9 3 40: 9 41: 9 42: 9 44: 3 44: 9 45: 46: 9 47:

Table 220: RERS sites in Human HC GLG FR1s where there are at least 20 GLGs cut

50: 9
There are 11 hits at base# 9

	BsoFI GCr	ngc		78				
5	1: 6	3: 6	6: 6	7:	6 8:	6	9:	6
	10: 6	15: 6	15: 67	16:	6 16:	67	17:	6
	17: 67	18: 6	18: 67	19:	6 19:	67	20:	6
	20: 67	21: 6	21: 67	22:	6 22:	67	23:	6
	23: 67	24: 6	24: 67	25:	6 25:	67	26:	6
10	26: 67	27: 6	27: 67	28:	6 28:	67	29:	6
	30: 6	30: 67	31: 6	31: 6	7 32:	6	32:	67
	33: 6	33: 67	34: 6	34: 6	7 35:	6	35:	67
	36: 6	36: 67	<u>37: 6</u>	37:	<u>9</u> <u>38:</u>	6	38:	9
	39: 6	39: 9	40: 3	40:	6 40:	9	41:	6
15	41: 9	42: 6	42: 9	43:	6 <u>44:</u>	3	44:	6
9	44: 9	<u>45: 6</u>	45: 9	<u>46:</u>	6 46:	<u>9</u>	<u>47:</u>	6
H-71	<u>47: 9</u>	48: 6	49: 6	50:	9 50:	12	51:	6
I :i	There ar	e 43 hi	ts at bas	e# 6 T	hese of	ten	occur	together.
4	There ar	e 11 hit	ts at bas	e# 9				
20 and and an an an an an	There ar	e 2 hit	s at bas	e# 3				
	There ar	e 21 hit	s at bas	e# 67				
	Tsel Gcwg	ic		78				
	1: 6	3: 6	6: 6	7:	6 8:	6	9:	6
± 25	10: 6	15: 6	15: 67	16:	6 16:	67	17:	6
	17: 67	18: 6	18: 67	19:	6 19:	67	20:	6
å	20: 67	21: 6	21: 67	22:	6 22:	67	23:	6
	23: 67	24: 6	24: 67	25:	6 25:	67	26:	6
	26: 67	27: 6	27: 67	28:	6 28:	67	29:	6
30	30: 6	30: 67	31: 6	31: 6	7 32:	6	32:	67
	33: 6	33: 67	34: 6	34: 6	7 35:	6	35:	67
	36: 6	36: 67	<u>37:</u> 6	37: 9	<u> 38:</u>	6	38:	<u>9</u>
	<u>39: 6</u>	39: 9	40: 3	40:	6 40:	9	41:	6
	<u>41: 9</u>	<u>42: 6</u>	42: 9	43:	6 <u>44:</u>	3	44:	6
35	44: 9	<u>45: 6</u>	<u>45: 9</u>	46:	6 46:	9	<u>47:</u>	6
	<u>47: 9</u>	48: 6	49: 6	50: 9	9 50:	12	51:	6
			_					•

There are 43 hits at base# 6 Often together.

There are 11 hits at base# 9

```
There are
                          1 hits at base# 12
           There are 21 hits at base# 67
     5
          MspAlI CMGckg
                                                48
             1:
                 7
                        3:
                            7
                                   4:
                                       7
                                              5:
                                                 7
                                                         6:
                                                             7
                                                                    7:
                                                                         7
             8:
                        9:
                            7
                                 10:
                                        7
                                            11:
                                                       15:
                                                             7
                                                                   16:
                                                                         7
           17:
                 7
                      18:
                            7
                                 19:
                                        7
                                                  7
                                                                         7
                                            20:
                                                       21:
                                                             7
                                                                  22:
           23:
                                 25:
                                       7
                 7
                      24:
                            7
                                            26:
                                                   7
                                                       27:
                                                             7
                                                                         7
                                                                  28:
    10
           29:
                 7
                            7
                                       7
                      30:
                                 31:
                                                  7
                                                             7
                                                                         7
                                             32:
                                                       33:
                                                                   34:
           35:
                 7
                      36:
                            7
                                 37:
                                       7
                                             38:
                                                  7
                                                             7
                                                       39:
                                                                   40:
           40:
                            7
                                        7
                                                             7
                                                                         7
                      41:
                                 42:
                                                                   45:
                                            44:
                                                       44:
           46:
                 7
                      47:
                            7
                                 48:
                                        7
                                             49:
                                                             7
                                                                        7
                                                       50:
                                                                   51:
           There are 46 hits at base#
    15
          PvuII CAGctg
                                                48
Ę
Ţ
             1:
                 7
                       3:
                            7
                                       7
                                  4:
                                              5:
                                                 7
                                                         6:
                                                                    7:
                                                                        7
Ö
            8:
                 7
                       9:
                            7
                                 10:
                                       7
                                            11:
                                                  7
                                                       15:
                                                             7
                                                                  16:
                                                                        7
W. III
           17:
                 7
                      18:
                            7
                                 19:
                                       7
                                            20:
                                                  7
                                                             7
                                                                  22:
                                                                         7
                                                       21:
20
           23:
                 7
                                       7
                      24:
                                 25:
                                            26:
                                                  7
                                                       27:
                                                                  28:
                                                                        7
                 7
           29:
                      30:
                            7
                                 31:
                                       7
                                            32:
                                                  7
                                                             7
                                                                  34:
                                                                        7
                                                       33:
Ħ
           35:
                 7
                                 37:
                      36:
                            7
                                       7
                                            38:
                                                  7
                                                       39:
                                                             7
                                                                  40:
                                                                        1
17
                                                       44:
                 7
                                            <u>44</u>:
           40:
                      41:
                                 42:
                                       7
                                                  1
                                                             7
                                                                  45:
                                                                        7
արտ արտ
անու հիս
           46:
                      47:
                            7
                                 48:
                                       7
                                            49:
                                                  7
                                                       50:
                                                             7
                                                                  51:
                                                                        7
    25
           There are 46 hits at base#
Ü
           There are
                          2 hits at base#
          AluI AGct
                                                54
            1:
                 8
                       2:
                            8
                                  3:
                                       8
                                              4:
                                                 8
                                                        4: 24
                                                                   5:
                                                                        8
    30
            6:
                       7:
                 8
                            8
                                  8:
                                       8
                                             9:
                                                       10: 8
                                                  8
                                                                  11:
                                                                        8
           15:
                 8
                      16:
                            8
                                 17:
                                       8
                                            18:
                                                  8
                                                       19:
                                                             8
                                                                  20:
                                                                        8
           21:
                 8
                      22:
                            8
                                 23:
                                       8
                                            24:
                                                  8
                                                       25:
                                                            8
                                                                  26:
                                                                        8
           27:
                 8
                      28:
                            8
                                 29:
                                       8
                                            29: 69
                                                       30:
                                                                  31:
                                                                        8
           32:
                      33:
                            8
                                 34:
                                       8
                                            35:
                                                                  37:
                 8
                                                  8
                                                       36:
                                                                        8
    35
           38:
                      39:
                                 40:
                                       2
                                            40:
                                                 8
                                                       41:
                                                                  42:
                                                                        8
           43:
                 8
                      <u>44:</u> 2
                                 44:
                                       8
                                            45:
                                                  8
                                                                  47:
                                                       46:
                                                             8
                                                                        8
```

2 hits at base#

There are

48:

48: 82

There are 48 hits at base# 8

49:

8

49: 82

50:

51:

8

There are 2 hits at base# 2

		DdeI	Ctna	ıg				4	48					
		1:	26	1:	48	2:	26	2:	48	3:	26	3:	48	
	5	4:	26	4:	48	5:	26	5:	48	6:	26	6:	48	
		7:	26	7:	48	8:	26	8:	48	9:	26	10:	26	
		11:	26	12:	85	13:	85	14:	85	15:	52	16:	52	
		17:	52	18:	52	19:	52	20:	52	21:	52	22:	52	
		23:	52	24:	52	25:	52	26:	52	27:	52	28:	52	
	10	29:	52	30:	52	31:	52	32:	52	33:	52	35:	30	
		35:	52	36:	52	40:	24	49:	52	51:	26	51:	48	
		The	re ar	e 22	hi 2	ts at	ba	se# 52	52	and 4	8 ne	ver t	oget:	her.
		The	re ar	e <u> </u>) hi	ts at	bas	se# 48						
		The	re ar	e 12	hi h	ts at	ba	se# 26	26	and 2	4 ne	ver t	oget:	her.
	15													
=		HphI	tcac	:c				4	12	•				
		1:	86	3:	86	6:	86	7:	86	8:	80	11:	86	
1		12:	5	13:	5	14:	5	15:	80	16:	80	17:	80	
4		18:	80	20:	80	21:	80	22:	80	23:	80	24:	80	
4. U	20	25:	80	26:	80	27:	80	28:	80	29:	80	30:	80	
1		31:	80	32:	80	33:	80	34:	80	35:	80	36:	80	
Π.		37:	59	38:	59	39:	59	40:	59	41:	59	42:	59	
11111		43:	59	44:	59	45:	59	46:	59	47:	59	50:	59	
11 11 11 11		The	re ar	e 22	hi?	ts at	bas	se# 80	80	and 8	6 ne	ver t	oget:	her
4	25	The	re az	e 5	hi	ts at	bas	se# 86						
₩ ₩ ₩ ₩		The	ce ar	e 12	? hi	ts at	bas	se# 59						
		BssKl	Ncc	ngg				į	50					
		1:	39	2:	39	3:	39	4:	39	5:	39	7:	39	
	<i>30</i>	8:	39	9:	39	10:	39	11:	39	15:	39	16:	39	
		17:	39	18:	39	19:	39	20:	39	21:	29	21:	39	
		22:	39	23:	39	24:	39	25:	39	26:	39	27:	39	
		28:	39	29:	39	30:	39	31:	39	32:	39	33:	39	
		34:	39	35:	19	35:	39	36:	39	37:	24	38:	24	
	<i>35</i>	39:	24	41:	24	42:	24	44:	24	45:	24	46:	24	
		47:	24	<u>48:</u>	39	48:	40	<u>49:</u>	39	49:	40	50:	24	
		50:	73	51:	39									
		The	re ar	e 35	hi	ts at	bas	se# 39	39	and 4	0 to	gethe	r tw	ice.

There are 2 hits at base# 40

```
BsaJI Ccnngg
                                          47
           1: 40
                                        4: 40
                                                           7: 40
                     2: 40
                              3: 40
                                                 5: 40
           8: 40
                     9: 40
                            9: 47
                                       10: 40
                                                10: 47
                                                          11: 40
     5
          15: 40
                    18: 40
                             19: 40
                                       20: 40
                                                21: 40
                                                          22: 40
          23: 40
                    24: 40
                             25: 40
                                       26: 40
                                                27: 40
                                                          28: 40
          29: 40
                    30: 40
                                       32: 40
                                                          35: 20
                             31: 40
                                                34: 40
          35: 40
                    36: 40
                             37: 24
                                       38: 24
                                                39: 24
                                                          41: 24
          42: 24
                    44: 24
                             45: 24
                                       46: 24
                                                47: 24
                                                          48: 40
    10
         48: 41
                    49: 40
                             49: 41
                                       50: 74
                                                51: 40
          There are 32 hits at base# 40 40 and 41 together twice
          There are
                       2 hits at base# 41
          There are
                       9 hits at base# 24
          There are
                       2 hits at base# 47
    15
         BstNI CCwgg
                                          44
O
PspGI ccwgg
         ScrFI($M.HpaII) CCwgg
           1: 40
                     2: 40
                              3: 40
                                        4: 40
                                                 5: 40
                                                           7: 40
    20
           8: 40
                                       11: 40
                                                15: 40
                     9: 40
                             10: 40
                                                          16: 40
Ü
          17: 40
                   18: 40
                                                          21: 40
                             19: 40
                                       20: 40
                                                21: 30
ţħ.
          22: 40
                    23: 40
                             24: 40
                                       25: 40
                                                          27: 40
                                                26: 40
28: 40
                    29: 40
                             30: 40
                                       31: 40
                                                32: 40
                                                          33: 40
          34: 40
                    35: 40
                             36: 40
                                       37: 25
                                                38: 25
                                                          39: 25
    25
          41: 25
                    42: 25
                             44: 25
                                       45: 25
                                                46: 25
                                                          47: 25
          50: 25
                    51: 40
13
          There are 33 hits at base# 40
         ScrFI CCngg
                                          50
    30
           1: 40
                     2: 40
                              3: 40
                                        4: 40
                                                  5: 40
                                                           7: 40
           8: 40
                     9: 40
                             10: 40
                                       11: 40
                                                15: 40
                                                          16: 40
          17: 40
                    18: 40
                             19: 40
                                       20: 40
                                                21: 30
                                                          21: 40
          22: 40
                    23: 40
                             24: 40
                                       25: 40
                                                26: 40
                                                          27: 40
          28: 40
                   29: 40
                                       31: 40
                             30: 40
                                                32: 40
                                                          33: 40
    35
          34: 40
                    35: 20
                             35: 40
                                       36: 40
                                                37: 25
                                                          38: 25
          39: 25
                    41: 25
                             42: 25
                                       44: 25
                                                45: 25
                                                          46: 25
          47: 25
                    48: 40
                             48: 41
                                       49: 40
                                                49: 41
                                                          50: 25
          50: 74
                    51: 40
          There are 35 hits at base# 40
```

There are 11 hits at base# 44

		Eco0109I	RGgnccy			3	34							•
		1: 43	2: 43	3:	43	4:	43	5: 43	6:	43				
	5	7: 43	8: 43	9:	43	10:	43	15: 46	16:	46				
		17: 46	18: 46	19:	46	20:	46	21: 46	22:	46				
		23: 46	24: 46	25:	46	26:	46	27: 46	28:	46			_	
		30: 46	31: 46	32:	46	33:	46	34: 46	35:	46	•			
		36: 46	37: 46	43:	79	51:	43							
	10	There a	re 22 hit	s at	bas	e# 46	46	and 43 ne	ever t	ogeth	er			
		There a	re 11 hit	s at	bas	e# 43								
		NlaIV GG	Nncc			-	71							
		1: 43	2: 43	3:	43	4:	43	5: 43	6:	43				
		7: 43	8: 43	9:	43	9:	79	10: 43	10:	79				
	15	15: 46	15: 47	16:	47	<u> 17:</u>	46	17: 47	18:	46	•			
T.		<u> 18: 47</u>	19: 46	19:	47	<u> 20:</u>	46	20: 47	<u>21:</u>	46				
dem den den den grap ger aleng dem den den den		21: 47	22: 46	22:	47	23:	47	24: 47	25:	47				
[9 [.]		26: 47	27: 46	27:	<u>47</u>	<u> 28:</u>	46	28: 47	29:	47				
*	•	30: 46	30: 47	<u>31:</u>	46	31:	47	32: 46	32:	47				
IJ	20	33: 46	33: 47	<u>34:</u>	46	34:	47	<u>35: 46</u>	35:	47				
Tan.		<u> 36: 46</u>	36: 47	37:	21	<u> 37:</u>	46	37: 47	37:	79				
E F. II.		38: 21	39: 21	39:	79	40:	79	41: 21	41:	79				
77		42: 21	42: 79	43:	79	44:	21	44: 79	45:	21				
==		45: 79	46: 21	46:	79	47:	21	51: 43						
the the the	25	There a	re 23 hit	s at	bas	e# 47	46	& 47 ofte	n tog	ether				
17		There a	re 17 hit	s at	bas	e# 46		There ar	e 11	hits	at l	oase#	43	
ļ4		Sau96I G	gncc			7	70							
		1: 44	2: 3	2:	44	3:	44	4: 44	5:	3	5:	44	6:	44
		7: 44	8: 22	8:	44	9:	44	10: 44	11:	3	12:	22	13:	22
	<i>30</i>	14: 22	15: 33	15:	47	16:	47	17: 47	18:	47	19:	47	20:	47
		21: 47	22: 47	23:	33	23:	47	24: 33	24:	47	25:	33	25:	47
		26: 33	26: 47	27:	47	28:	47	29: 47	30:	47	31:	33	31:	47
		32: 33	32: 47	33:	33	33:	47	34: 33	34:	47	35:	47	36:	47
		37: 21	37: 22	37:	47	<u> 38:</u>	21	38: 22	39:	21	39:	22	41:	21
	35	41: 22	42: 21	42:	22	43:	80	44: 21	44:	22	45:	21	45:	22
		46: 21	46: 22	47:	21	47:	22	50: 22	51:	44				
		There a	re 23 hit	s at	base	e# 4 7	Th€	se do not	occu:	r tog	ether	r.		

There are 14 hits at base# 22 These do occur together.
There are 9 hits at base# 21

	BsmA.		22									
5	1:	58	3:	58	4:	58	5:	58	8:	58	9:	58
	10:	58	13:	70	36:	18	37:	70	38:	70	39:	70
	40:	70	41:	70	42:	70	44:	70	45:	70	46:	70
	47:	70	48:	48	49:	48	50:	85				-
	There are 11 hits at base# 70											
10												
	-"-	27										
	13:	40	15:	48	16:	48	17:	48	18:	48	20:	48
	21:	48	22:	48	23:	48	24:	48	25:	48	26:	48
	27:	48	28:	48	29:	48	30:	10	30:	48	31:	48
15	32:	48	33:	48	35:	48	36:	48	43:	40	44:	40
	45:	40	46:	40	47:	40						
There are 20 hits at base# 48												
	AvaII Ggwcc					44						
<i>20</i>	Sau96I(\$M.HaeIII) Ggwcc 44											
	2:	3	5:	3	6:	44	8:	44	9:	44	10:	44
	11:	3	12:	22	13:	22	14:	22	15:	33	15:	47
	16:	47	17:	47	18:	47	19:	47	20:	47	21:	47
	22:	47	23:	33	23:	47	24:	33	24:	47	25:	33
25	25:	47	26:	33	26:	47	27:	47	28:	47	29:	47
	30:	47	31:	33	31:	47	32:	33	32:	47	33:	33
	33:	47	34:	33	34:	47	35:	47	36:	47	37:	47
	43:	80	50:	22								
	The	ce ar	e 23	3 hit	s at	base	4 47	44	& 47 r	never	toge	ether
<i>30</i>	The	ce ar	e 4	1 hit	s at	base	4 4 4					
	PpuM1	27										
	6:	43	8:	43	9:	43	10:	43	15:	46	16:	46
	17:	46	18:	46	19:	46	20:	46	21:	46	22:	46
35	23:	46	24:	46	25:	46	26:	46	27:	46	28:	46
	30:	46	31:	46	32:	46	33:	46	34:	46	35:	46
	36:	46	37:	46	43:	79						
	Ther	e are	e 22	hit?	s at	base	46	43	and 46	neve	er oc	ccur together.

There are 4 hits at base# 43

```
BsmFI GGGAC
                                       3
       8: 43
                37: 46
                          50: 77
     -"-
           gtccc
                                      33
5
                                    1: 0
      15: 48
                16: 48
                         17: 48
                                              1: 0
                                                      20: 48
      21: 48
                22: 48
                         23: 48
                                   24: 48
                                             25: 48
                                                      26: 48
      27: 48
                28: 48
                         29: 48
                                   30: 48
                                             31: 48
                                                      32: 48
                         35: 48
      33: 48
                34: 48
                                   36: 48
                                             37: 54
                                                      38: 54
      39: 54
                40: 54
                         41: 54
                                   42: 54
                                             43: 54
                                                      44: 54
10
      45: 54
                46: 54
                         47: 54
      There are 20 hits at base# 48
      There are 11 hits at base# 54
     HinfI Gantc
                                      80
15
       8: 77
                12: 16
                         13: 16
                                   14: 16
                                             15: 16
                                                      15: 56
      15: 77
                16: 16
                         16: 56
                                   16: 77
                                             17: 16
                                                      17: 56
      17: 77
                18: 16
                         18: 56
                                   18: 77
                                             19: 16
                                                      19: 56
      19: 77
                20: 16
                         20: 56
                                   20: 77
                                             21: 16
                                                      21: 56
      21: 77
                22: 16
                         22: 56
                                   22: 77
                                             23: 16
                                                      23: 56
20
      23: 77
                24: 16
                         24: 56
                                   24: 77
                                                      25: 56
                                             25: 16
      25: 77
               26: 16
                         26: 56
                                   26: 77
                                            27: 16
                                                      27: 26
      27: 56
               27: 77
                         28: 16
                                   28: 56
                                             28: 77
                                                      29: 16
      29: 56
               29: 77
                         30: 56
                                   31: 16
                                             31: 56
                                                      31: 77
      32: 16
                32: 56
                         32: 77
                                   33: 16
                                             33: 56
                                                      33: 77
25
      34: 16
                35: 16
                         35: 56
                                   35: 77
                                             36: 16
                                                      36: 26
      36: 56
                36: 77
                         37: 16
                                   38: 16
                                             39: 16
                                                      40: 16
      41: 16
                42: 16
                         44: 16
                                   45: 16
                                             46: 16
                                                      47: 16
      48: 46
                49: 46
      There are 34 hits at base# 16
30
     Tfil Gawtc
                                      21
       8: 77
               15: 77
                         16: 77
                                   17: 77
                                            18: 77
                                                      19: 77
      20: 77
               21: 77
                         22: 77
                                   23: 77
                                             24: 77
                                                      25: 77
      26: 77
               27: 77
                         28: 77
                                   29: 77
                                            31: 77
                                                      32: 77
35
      33: 77
                35: 77
                         36: 77
```

There are 21 hits at base# 77

tat tim the time that tall tall

Į.

Ļ±,

Indiana

```
MlyI GAGTC
                                          38
          12: 16
                   13: 16
                             14: 16
                                       15: 16
                                                16: 16
                                                          17: 16
          18: 16
                    19: 16
                             20: 16
                                       21: 16
                                                22: 16
                                                          23: 16
          24: 16
                   25: 16
                             26: 16
                                       27: 16
                                                27: 26
                                                          28: 16
     5
          29: 16
                   31: 16
                             32: 16
                                       33: 16
                                                34: 16
                                                          35: 16
          36: 16
                   36: 26
                             37: 16
                                       38: 16
                                                39: 16
                                                          40: 16
          41: 16
                    42: 16
                             44: 16
                                       45: 16
                                                46: 16
                                                          47: 16
          48: 46
                    49: 46
          There are 34 hits at base# 16
    10
         -"- GACTC
                                          21
          15: 56
                   16: 56
                             17: 56
                                       18: 56
                                                19: 56
                                                          20: 56
          21: 56
                   22: 56
                             23: 56
                                       24: 56
                                                25: 56
                                                          26: 56
                   28: 56
          27: 56
                             29: 56
                                       30: 56
                                                31: 56
                                                          32: 56
   15
          33: 56
                   35: 56
                             36: 56
          There are 21 hits at base# 56
[]
įĴ
(O
         PleI gagtc
                                          38
Ų
          12: 16
                   13: 16
                             14: 16
                                       15: 16
                                                16: 16
                                                          17: 16
'n.
   20
U
          18: 16
                   19: 16
                             20: 16
                                       21: 16
                                                22: 16
                                                          23: 16
13
          24: 16
                   25: 16
                                                          28: 16
                             26: 16
                                       27: 16
                                                27: 26
[]
          29: 16
                   31: 16
                             32: 16
                                       33: 16
                                                34: 16
                                                          35: 16
1 1 1 1 1 1
          36: 16
                   36: 26
                             37: 16
                                       38: 16
                                                39: 16
                                                          40: 16
          41: 16
                   42: 16
                             44: 16
                                       45: 16
                                                46: 16
                                                          47: 16
   25
          48: 46
                   49: 46
          There are 34 hits at base# 16
(J
         -"- gactc
                                          21
          15: 56
                   16: 56
                             17: 56
                                       18: 56
                                                19: 56
                                                          20: 56
          21: 56
                   22: 56
                             23: 56
                                      24: 56
                                                25: 56
                                                          26: 56
   30
          27: 56
                   28: 56
                             29: 56
                                       30: 56
                                                31: 56
                                                          32: 56
          33: 56
                   35: 56
                             36: 56
          There are 21 hits at base# 56
         AlwNI CAGNNNctq
                                          26
         15: 68
                   16: 68
                             17: 68
                                      18: 68
                                                19: 68
                                                         20: 68
   35
         21: 68
                   22: 68
                             23: 68
                                      24: 68
                                                25: 68
                                                         26: 68
          27: 68
                   28: 68
                             29: 68
                                      30: 68
                                                31: 68
                                                          32: 68
          33: 68
                   34: 68
                             35: 68
                                      36: 68
                                                39: 46
                                                          40: 46
          41: 46
                   42: 46
```

=

There are 22 hits at base# 68

Table 255: Analysis of frequency of matching REdaptors in actual V genes A: HpyCH4V in HC at bases 35-56

٠:

Seqs with the expected RE site only......1004

(Counts only cases with 4 or fewer mismatches)

Seqs with only an unexpected site..... 0

Seqs with both expected and unexpected....

48

(Counts only cases with 4 or fewer mismatches)

S

Seqs with no sites..... 0

B: BlpI in HC

		601												
tccttaccatgaccaacatgga	2-26	0	0	0	0	0	0	0	0	2	0	8	14	
tccttacaatgaccaacatgga	2-70	0	0	0	0	0	1	2	8	æ	. 15	28	13	
ccctgcagctgaactctgtgac	6-1	ш	0	Н	ω	1	1	0	1	ω	6	16	12	
ccctgaagctgagctctgtgac	4301	467	-	4	4	10	21	38	81	78	249	486	11	20
atcttcaaatgggcagcctgag	3-64	0	0	0	0	0	0	0	1	0	י	2	10	
atcttcaaatgaacagcctgag	3-66	0	0	0	0	0	0	ب	2	2	18	23	9	
atctgcagatctgcagcctaaa	74.1	0	0	0	0	0	0	_	0	N	0	ω	80	
atctgcaaatgaacagtctgag	3-20	0	0	0	0	ω	۳	12	25	16	25	82	7	
atctgcaaatgaacagcctgag	3303	0	0	\vdash	0	ω	σ	15	41	88	186	340	Q	15
atctgcaaatgaacagcctgaa	3-15	0	0	0	0	Н	ω	17	10	11	13	55	5	
acctgcagtggagcagcctgaa	5-51	2	0	۲	L	μ	9	10	16	32	50	120	4	
acatggagctgaggagcctgag	1-18	0	0	0	0	0	r	σ	8	80	17	34	ω	
acatgga gctgagc aggctgag	1-02	12	\vdash	0	μ.	0	0	0	0	۲	11	14	2	
acatggaGCTGAGCagcctgag	1-58	119	0	4	-	9	Q	13	11	16	73	133	щ	10
	Name	Ncut	۵	7	6	5	4	ω	2	L	0	Ntot	Id	

	Name	Full sequence	Dot mode
	1-58	acatggaGCTGAGCagcctgag	acatggaGCTGAGCagcctgag
	1-02	acatgga gctgagc aggctgag	
	1-18	acatggagctgaggagcctgag	gg
Ŋ	5-51	acctgcagtggagcagcctgaa	ctga
	3-15	atctgcaaatgaacagcctgaa	.tcc.aaaa
	3-30.3	atctgcaaatgaacagcctgag	.tcc.aaa
,	3-20	atctgcaaatgaacagtctgag	.tcc.aaat
	7-4.1	atctgcagatctgcagcctaaa	.tcca.cta.a
10	3-66	atcttcaaatgaacagcctgag	.tc.tc.aaa
	3-64	atcttcaaatgggcagcctgag	.tc.tc.aag
	4-30.1	ccctgaagctgagctctgtgac	c.catctgc
	6-1	ccctgcagctgaactctgtgac	c.cca.tctgc
	2-70	tccttacaatgaccaacatgga	t.c.tacaaca.aga
15	2-26	tccttaccatgaccaacatgga	t.c.taccaca.aga
	Seqs with	the expected RE site on	site only 597 (counting sequences with 4 or fewer mismatches)
	Seqs with	only an unexpected	site 2
	Seqs with	both expected and unexpected	ected 2
20	Seqs with	no sites	686

C: HpyCH4III, Bst4CI, or Taal in HC

In scoring whether the RE site of interest is present, only ONs that have 4 or fewer mismatches are counted.

25 Number of sequences...... 1617

			25					20					15					7	,				Ç				
Seqs Seqs	Cumul	Group	24	23	22	21	20	19	18	17	16	15	14	13	12	11	10	9	œ	7	0	տ	4.	ω	2	 -	Id
Seqs with the expo	Cumulative		213	40	75	17	34	2	–	2	14	75	117	ω	18	35	23	7	9	23	15	.4	16	173	457	244	Ntot
expe y an	337	337	26	14	15	5	4.	0	0	0	2	21	29	1	2	5	7	۳	 -	.4.	۲	0	0	52	69	78	0
cted RE si	808	471	56	15	17	4	9	0	0	0	2	25	23	2	ω	10	ω	ω	ب	8	0	0	ω	45	150	92	 -
E site	1171	363	60	4	24	2	9	0	1	_	2	13	28	0	2	7	ر د	٦	ш			–	2	36	115	43	2
te only site	1389	218	42	ъ	7	2	4	0	. 0	0	ω	9	22	0	2	o	5	_	0	2	0	0	2	22	66	18	ω
: :	1519	130	20	-	10	ω	Մ	0	0	0	0	H	8	0	6	ω	2	0	ω	2	6	1	→	14	34	10	4
1511	1577	58	7	0	_	_	ω	0	0	1	ω	4	4	0	_	ω	1	0	2	سا	4.	<u> </u>	6	ω	11	_	5
	1600	23	2	_	_	0	0	0	0	0	Н	N	8	0	0	0	0	1		1	1	0	0	0	8	2	6
	1611	11	0	0	0	0	0	0	0	0	–	0	–	0	2	_	0	0	0	0	بر	–	–	0	w	0	7
	1617	6	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	بسر	1	_	0	l 80
			204	39	73	16	31	0	1	1	9	69	110	ω	15	31	22	6	6	21	æ	2	œ	169	434	241	Ncut
			5a	55	43	43	42	3 d	37	37	34	33	32	32	31	31	30	27	22	20	15	14	12	10	10	10	1
			5a#49	551#48	439#44	4302#40	428#38	3d#36	373#34	372#33	349#29	330#23,	323#22	320#20	315#19	313#18,	309#16,	270#14	226#13	205#12	158#8	145#6	124#5,1	108#3	103#2,3	102#1,1	
			ccatgtattactgtgcgagaAA	ccatgtattactgtgcgagaca	ctgtgtattactgtgcgagaca	ccgtgtattactgtgccagaga	ccgtgtattactgtgcgagaaa	ctgtgtattactgtaagaaaga	ccgtgtattactgtactagaca	ccgtgtattactgtgctagaga	ccgtgtattactgtactagaga	ctgtgtattactgtgcgaaaga	ccgtatattactgtgcgaaaga	ccttgtatcactgtgcgagaga	ccgtgtattactgtaccacaga	ctgtgtattactgtgcaagaga	ccttgtattactgtgcaaaaga	ccacgtattactgtgcacggat	ccacatattactgtgcacggat	ccacatattactgtgcacacag	ccgtgtattactgtgcggcaga	ccatgtattactgtgcaagata	ccgtgtattactgtgcaacaga	ccgtgtattactgtgcgagagg	ctgtgtattactgtgcgagaga	ccgtgtattACTGTgcgagaga	acngt
			a	a	.tc.	c	a .	.taaa	a.tc.	· · · · · · · · · · · · · · · · · · ·	a.t	.ta		tc	a.c.c.	. t	t	acac.gat	acaac.gat	acaacacag	gc	at.	a.c			ccgtgtattactgtgcgagaga	acnqt

Seqs with both expected and unexpected.... 8

		Se	eqs with	no	sites	• • • • •	• • • •	• • • • • •	· · · ·	• • • •	0				
		An	alysis	rep	peate	ed us	ing	only	8 k	est	REda	ptors	5		
	5	Id	Ntot	0	1	2	3	4	5	6	7	. 8+			
		1	301	78	101	54	32	16	9	10	1	0	281	102#1	ccgtgtattactgtgcgagaga
		2	493	69	155	125	73	37	14	11	3	6	459	103#2	ctgtgtattactgtgcgagaga
		3	189	52	45	38	23	18	5	4	1	3	176	108#3	ccgtgtattactgtgcgagagg
		4	127	29	23	28	24	10	6	5	2	0	114	323#22	ccgtatattactgtgcgaaaga
	10	5	78	21	25	14	11	1	4	2	0	0	72	330#23	ctgtgtattactgtgcgaaaga
		6	79	15	17	25	8	11	1	2	0	0	76	439#44	ctgtgtattactgtgcgagaca
		7	43	14	15	5	5	3	0	1	0	0	42	551#48	ccatgtattactgtgcgagaca
		8	307	26	63	72	51	38	24	14	13	6	250	5a#49	ccatgtattactgtgcgaga
		1	102#	1	ccg	gtgta	tta	ctgtg	cgaç	gaga	ccg	tgtat	tact	tgtgcgaga	ıga
	15	2	103#	2	ctç	gtgta	tta	ctgtg	cgaç	gaga	.t.	• • • •			••
		3	108#	3	ccc	gtgta	tta	ctgtg	cgaç	gagg					·g
47. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1.		4	323#	22	ccg	gtata	tta	ctgtg	cgaa	aga		.a		a.	••
ta ta		5	330#	23	cto	gtgta	tta	ctgtg	cgaa	aga	.t.			a.	••
W.		6	439#	44	cto	gtgta	tta	ctgtg	cgaç	jaca	.t.	• • • •			с.
'n j	<i>20</i>	7	551#	48	cca	tgta	tta	ctgtg	cgaç	jaca	a				с.
4H		8	5a#4	9	cca	ıtgta	ttad	ctgtg	cgaç	jaAA	a				AA
in.															
8		Se	eqs wi	th t	the e	хрес	ted	RE s	ite	only	7	14	163 /	/ 1617	
T]		Se	eqs wi	th o	only	an u	nex	pecte	d si	te.			0		
== }±	25	Se	eqs wi	th k	ooth	expe	cte	d and	une	expe	cted.		7		
ٿِي ۴		Se	eqs wi	th r	no si	tes.	• • •						0		
				-											
ļ.ā	*														

GCA TCT ACA GGA GAC AGA GTC ACC ATC ACT TGT

GTC ATC TGG ATG ACC CAG TCT CCA TCC TTA CTC TCT

L9

		GCA	TCT	ACA	GGA	GAC	AGA	GTC	ACC	ATC	AGT	TGT	!	L24
		GCC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	
		GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGC	!	L11
		GAC	ATC	CAG	ATG	ACC	CAG	TCT	CCT	TCC	ACC	CTG	TCT	
	5	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGC	!	L12
		GAT	ATT	GTG	ATG	ACC	CAG	ACT	CCA	CTC	TCC	CTG	CCC	
		GTC	ACC	CCT	GGA	GAG	CCG	GCC	TCC	ATC	TCC	TGC	!	011
		GAT	ATT	GTG	ATG	ACC	CAG	ACT	CCA	CTC	TCC	CTG	CCC	
		GTC	ACC	CCT	GGA	GAG	CCG	GCC	TCC	ATC	TCC	TGC	!	01
	10	GAT	GTT	GTG	ATG	ACT	CAG	TCT	CCA	CTC	TCC	CTG	CCC	
		GTC	ACC	CTT	GGA	CAG	CCG	GCC	TCC	ATC	TCC	TGC	!	A17
		GAT	GTT	GTG	ATG	ACT	CAG	TCT	CCA	CTC	TCC	CTG	CCC	
		GTC	ACC	CTT	GGA	CAG	·CCG	GCC	TCC	ATC	TCC	TGC	!	A1
		GAT	ATT	GTG	ATG	ACC	CAG	ACT	CCA	CTC	TCT	CTG	TCC	
4.2	<i>15</i>	GTC	ACC	CCT	GGA	CAG	CCG	GCC	TCC	ATC	TCC	TGC	!	A18
Į.		GAT	ATT	GTG	ATG	ACC	CAG	ACT	CCA	CTC	TCT	CTG	TCC	
lj		GTC	ACC	CCT	GGA	CAG	CCG	GCC	TCC	ATC	TCC	TGC	!	A2
`*J.		GAT	ATT	GTG	ATG	ACT	CAG	TCT	CCA	CTC	TCC	CTG	CCC	
IJ		GTC	ACC	CCT	GGA	GAG	CCG	GCC	TCC	ATC	TCC	TGC	!	A19
O O	20	GAT	ATT	GTG	ATG	ACT	CAG	TCT	CCA	CTC	TCC	CTG	CCC	
8		GTC	ACC	CCT	GGA	GAG	CCG	GCC	TCC	ATC	TCC	TGC	!	A3
13		GAT	ATT	GTG	ATG	ACC	CAG	ACT	CCA	CTC	TCC	TCA	CCT	
14	•	GTC	ACC	CTT	GGA	CAG	CCG	GCC	TCC	ATC	TCC	TGC	!	A23
, ii		GAA	ATT	GTG	TTG	ACG	CAG	TCT	CCA	GGC	ACC	CTG	TCT	
11	25	TTG	TCT	CCA	GGG	GAA	AGA	GCC	ACC	CTC	TCC	TGC	!	A27
5 -		GAA	ATT	GTG	TTG	ACG	CAG	TCT	CCA	GCC	ACC	CTG	TCT	
		TTG	TCT	CCA	GGG	GAA	AGA	GCC	ACC	CTC	TCC	TGC	!	A11
		GAA	ATA	GTG	ATG	ACG	CAG	TCT	CCA	GCC	ACC	CTG	TCT	
		GTG	TCT	CCA	GGG	GAA	AGA	GCC	ACC	CTC	TCC	TGC	1	L2
	30	GAA	ATA	GTG	ATG	ACG	CAG	TCT	CCA	GCC	ACC	CTG	TCT	
		GTG	TCT	CCA	GGG	GAA	AGA	GCC	ACC	CTC	TCC	TGC	!	L16
		GAA	ATT	GTG	TTG	ACA	CAG	TCT	CCA	GCC	ACC	CTG	TCT	
					GGG								!	L6
					TTG								TCT	
	<i>35</i>	TTG	TCT	CCA	GGG	GAA	AGA	GCC	ACC	CTC	TCC	TGC	!	L20
					ATG								TCT	

	TTG	TCT	CCA	GGG	GAA	AGA	GCC	ACC	CTC	TCC	TGC	!	L25
	GAC	ATC	GTG	ATG	ACC	CAG	TCT	CCA	GAC	TCC	CTG	GCT	
	GTG	TCT	CTG	GGC	GAG	AGG	GCC	ACC	ATC	AAC	TGC	!	В3
	GAA	ACG	ACA	CTC	ACG	CAG	TCT	CCA	GCA	TTC	ATG	TCA	
5	GCG	ACT	CCA	GGA	GAC	AAA	GTC	AAC	ATC	TCC	TGC	!	B2
	GAA	ATT	GTG	CTG	ACT	CAG	TCT	CCA	GAC	TTT	CAG	TCT	
	GTG	ACT	CCA	AAG	GAG	AAA	GTC	ACC	ATC	ACC	TGC	!	A26
	GAA	ATT	GTG	CTG	ACT	CAG	TCT	CCA	GAC	TTT	CAG	TCT	
	GTG	ACT	CCA	AAG	GAG	AAA	GTC	ACC	ATC	ACC	TGC	!	A10
10	GAT	GTT	GTG	ATG	ACA	CAG	TCT	CCA	GCT	TTC	CTC	TCT	
	GTG	ACT	CCA	GGG	GAG	AAA	GTC	ACC	ATC	ACC	TGC	!	A14

He strip to the street was street that the street of the s

Table 302 RERS sites found in Human Kappa FR1 GLGs

		MslI	FokI	PflFI	BsrI	BsmAI	Mnll	Нрусн 4V
עצו								
012	1-69	3	3 23	12 49	15	18 47	26	36
02	101-169	103	103 123	112 149	115	118 147	126	136
018	201-269	203	203 223	212 249	215	218 247	226	236
90	301-369	303	303 323	312 349	315	318 347	326	336
A20	401-469	403	403 423	412 449	415	418 447	426	436
A30	501-569	503	503 523	512 549	515	518 547	526	536
114	601-669	603	603	612 649	615	618 647	-	636
L1	701-769	703	703 723	712 749	715	718 747	726	736
L15	801-869	803	803 823	812 849	815	818 847	826	836
L4	901-969	-	903 923	912 949	906 915	918 947	926	936
L18	1001-1069	· 1	1003	1012 1049	1006 1015	1018 1047	1026	1036
1.5	1101-1169	1103	1	1112 1149	1115	1118 1147	-	1136
L19	1201-1269	1203	1203	1212 1249	1215	1218 1247	-	1236
L8	1301-1369	1	1303 1323	1312 1349	1306 1315	1318 1347	_	1336
123	1401-1469	1403	1403 1408	1412 1449	1415	1418 1447	1	1436
1.9	1501-1569	1503	1503 1508 1523	1512 1549	1515	1518 1547	1526	1536
L24	1601-1669	1603	1608 1623	1612 1649	1615	1618 1647	1	1636
L11	1701-1769	1703	1703 1723	1712 1749	1715	1718 1747	1726	1736
112	1801-1869	1803	1803	1812 1849	1815	1818 1847	t	1836

The Hall Lies, the R.S. Hall State 10 Hall State 11 Hall State Class Cla

		1:::::::	:	1	Ι	1	1	Γ	r			.							· · · · ·			
Нрусн	40		-	1	ι	1	ι	ı	1	-	1		1		1		ı		ı		ı	
MnlI			1956	2056	2156	2256	2356	2456	2556	2656	2729 2756			2860		2960		3060		3160		3260
BsmAI			-		2118	2218	-	ı	2518	2618	-		2818 2839		2918 2939		3018 3039		3118 3139		3218 3239	
BsrI			-	1	ł	1	ŀ	-	•	-	_		•		_		-		-		I	
PflFI			-	-	2112	2212	_	_	2512	2612	_		2812		2912		3012		3112		3212	
FokI	< <		ı	-	_	_	-	_	-	1	-		-		ı		ı		1			
MslI			-	_	_	-	_	_	_	-	_		1		-		ı		ı		-	
			1901-1969	2001-2069	2101-2169	2201-2269	2301-2369	2401-2469	2501-2569	2601-2669	2701-2769	1	2801-2869		2901-2969		3001-3069		3101-3169		3201-3269	
		VKII	011	01	A17	A1	A18	A2	A19	A3	A23	VKIII	A27		A11		Г.2		L16		T.6	

-									
			MslI	FokI	PflFI	BsrI	BsmAI	MnlI	Нрусн
				<> <		,			40
	L20	3301-3369	1	ſ	3312	-	3318 3339		
								3360	
	125	3401-3469	ı	1	3412	-	3418 3439		ı
								3460	
	VKIV								
	B3	3501-3569	3503	ı	3512	3515	3518 3539	,	_
								3551<	
'n	VKV								
	B2	3601-3669	1	-	3649	-	3618 3647		ı
	VKVI								
	A26	3701-3769	1	1	3712	-	3718		ı
	A10	3801-3869	ı		3812		3818		ı
01	A14	3901-3969	ı	1	3912	ı	3918	3930>	ı

Table 302 RERS sites found in Human Kappa FR1 GLGs, continued

		SfaNI	SfcI	SfcI HinfI	MlyI	MaeIII	HphI	Hpall
					>	Tsp45I	xx38 xx56 xx62 MspI	MspI
						same sites		xx06 xx52
VKI								
012	1-69	37	41	53	53	55	56	1
02 1	101-169	137	141	153	153	155	156	ı
018 2	201-269	237	241	253	253	255	256	1

> < Tsp451 same sites 353 355 453 455 453 455 653 655 653 655 653 655 1053 1055 1153 1155 1153 1155 1153 1155 1153 1155 1153 1155 1153 1155 1153 1155 1153 1155 1153 1155 1153 1155 1153 1155 1153 1155 2018 2037 2018 2037		:	SfaNI	sfcI	HinfI	міуі	MaeIII	IdphI	Hpall
301-369 337 341 353 353 355 355 401-469 437 441 453 453 455 455 501-569 537 541 453 553 555 555 601-669 637 641 653 653 655 555 601-669 637 641 653 653 655 755 801-869 637 641 653 653 655 755 1001-1069 1037 1441 1053 1053 1055 1155 1101-1169 1037 1441 1153 1153 1155 1155 1101-1469 1337 1441 1153 1153 1155 1155 1101-1469 1337 1441 1153 1153 1155 1155 1101-1469 1337 1441 1153 1153 1155 1155 1101-1469 1337 1441 11653 11653						· 	Tsp45I	xx38 xx56 xx62	MspI
301-369 337 341 353 353 355 355 445 455									xx06 xx52
401-469 437 441 453 453 455 455 555 455 555	90	301-369	337	341	353	353	355	356	1
501-569 537 541 553 553 555 555 655 655 655 655 655 655 655 655 655 655 655 655 655 655 655 655 737 741 753 753 755	A20	401-469	437	441	453	453	455	456	-
601-669 637 641 653 653 655 755 771 741 753 655 755	A30	501-569	537	541	553	553	555	556	1
701-769 737 741 753 755 755 801-869 837 841 853 755 855 855 901-969 937 941 953 953 955 955 1001-1069 1037 1041 1053 1053 1055 1055 1101-1169 1137 1141 1153 1153 1155 1155 1301-1369 1337 1341 1453 1453 1455 1355 1401-1469 1337 1441 1453 1453 1455 1555 1501-1569 1537 1541 1553 1553 1555 1555 1501-1569 1537 1641 1553 1553 1555 1555 1801-1869 1837 1841 1653 1655 1655 1755 1801-1869	L14	601-669	637	641	653	653	655	656	1
801-869 837 841 853 853 855	171	701-769	737	741	753	753	755	756	-
901-969 937 941 953 955 955 1001-1069 1037 1041 1053 1053 1055 1055 1101-1169 1137 1141 1153 1155 1155 1155 1201-1269 1237 1241 1253 1253 1255 1255 1301-1369 1337 1341 1453 1453 1455 1455 1401-1469 1437 1441 1553 1553 1455 1455 1501-1569 1637 1741 1753 1653 1655 1755 1601-1669 1637 1741 1753 1755 1755 1755 1801-1869 1837 1841 1753 1853 1853 1855 2001-2069 - 2018 2018 2037 2038 2101-2169 - 2112 2112 2112 2118	L15	801-869	837	841	853	853	855	856	ı
1001-1069 1037 1041 1053 1053 1055 1055 1101-1169 1137 1141 1153 1153 1155 1155 1201-1269 1237 1241 1253 1253 1253 1255 1301-1369 1337 1341 1453 1453 1455 1455 1401-1469 1437 1441 1553 1553 1655 1655 1601-1669 1637 1641 1653 1653 1655 1655 1801-1869 1637 1641 1653 1655 1755 1755 1801-1869 1637 1641 1653 1653 1655 1755 1901-1869 - - 1918 1918 1937 1938 2001-2069 - - 2018 2137 2138 201-2269 - - 2122 2212 2237 2237 2238	L4	901-969	937	941	953	953	955	926	-
1201-1169 1137 1141 1153 1155	L18	1001-1069	1037	1041	1053	1053	1055	1056	ı
1201-1269 1237 1241 1253 1255 1355 1355 1355 1355 1355 1355 1355 1355 1355 1355 1355 1355 1455 1455 1455 1455 1455 1455 1455 1455 1455 1455 1455 1455 1455 1556 1556	1.5	1101-1169	1137	1141	1153	1153	1155	1156	ı
1301-1369 1337 1341 1353 1355 1355 1401-1469 1437 1441 1453 1453 1455 1455 1501-1569 1537 1541 1553 1553 1555 1555 1601-1669 1637 1641 1653 1655 1755 1755 1701-1769 1737 1741 1753 1753 1755 1755 1801-1869 1837 1841 1853 1855 1835 1901-1969 - - 1918 1918 1937 1938 2001-2069 - - 2018 2018 2037 2038 2101-2169 - - 2112 212 2237 2238	L19	1201-1269	1237	1241	1253	1253	1255	1256	-
1401-1469 1437 1441 1453 1453 1455 1455 1555 1555 1555 1555 1555 1555 1555 1555 1555 1555 1555 1555 1555 1555 1555 1555 1555 1755 1855 1855 1855 1855 1855 1834 1855 1834	1.8	1301-1369	1337	1341	1353	1353	1355	1356	-
1501-1569 1537 1541 1553 1555 1555 1555 1555 1555 1555 1555 1555 1555 1555 1555 1555 1555 1555 1555 1555 1555 1555 1755	L23	1401-1469	1437	1441	1453	1453	1455	1456	1406
1601–1669 1637 1641 1653 1655 1655 1655 1655 1655 1750 1751 1753 1753 1755	1.9	1501-1569	1537	1541	1553	1553	1555	1556	1506
1701-1769 1737 1741 1753 1755 1755 1801-1869 1837 1841 1853 1855 1855 1901-1969 - - 1918 1937 1938 2001-2069 - - 2018 2037 2038 2101-2169 - - 2112 2137 2138 2201-2269 - - 2212 2212 2237 2238	L24	1601-1669	1637	1641	1653	1653	1655	1656	
1801–1869 1837 1841 1853 1855 1855 1855 1855 1855 1855 1855 1855 1855 1855 1855 1855 1855 1855 1855 1855 1856 1857 1858	L11	1701-1769	1737	1741	1753	1753	1755	1756	
1901–1969 - - 1918 1918 1937 2001–2069 - - 2018 2018 2037 2101–2169 - - 2112 2137 2201–2269 - - 2212 2237	L12	1801-1869	1837	1841	1853	1853	1855	1856	
1901–1969 - - 1918 1937 2001–2069 - - 2018 2037 2101–2169 - - 2112 2137 2201–2269 - - 2212 2237	VKII								
2001–2069 - - 2018 2037 2101–2169 - - 2112 2137 2201–2269 - - 2212 2237	011	1901-1969	-	ı	1918	1918	1937	1938	1952
2101-2169 - - 2112 2137 2201-2269 - - 2212 2212	01	2001-2069	,	ı	2018	2018	2037	2038	202
2201-2269 2212 2237	A17	2101-2169	I	1	2112	2112	2137	2138	2152
	A1	2201-2269	1	1	2212	2212	2237	2238	2252

the first the man and a man that the the second of the transition of the first the color of the color of the first the color of the color of the first the color of the color of the first the color of the first the color of the color of the first the color of the first the color of the color

		SfaNI	SfcI	HinfI	MlyI	MaeIII	HphI	Hpall
					>	Tsp45I	xx38 xx56 xx62	MspI
						same sites		xx06 xx52
A18	2301-2369	ı	1	2318	2318	2337	2338	2352
A2	2401-2469	1	1	2418	2418	2437	2438	2452
A19	2501-2569	1	1	2512	2512	2537	2538	2552
A3	2601-2669		-	2612	2612	2637	2638	2652
A23	2701-2769	-	1	2718	2718	2737	2731* 2738*	_
VKIII	1							
A27	2801-2869	_	ı	_				-
A11	2901-2969	_	ı	ŧ	1			_
1.2	3001-3069	_	1	1	1			-
L16	3101-3169	- -	ł	-	1			-
91	3201-3269	-	1	1	_			-
120	3301-3369	_	-	1	_			_
L25	3401-3469		-	1	1			_
VKIV								
В3	3501-3569	-	-	3525	3525			-
VRV								
B2	3601-3669	ı	1	3639	3639			-
LVNV								
A26	3701-3769	1	ţ	3712 3739	3712 3739	3737 3755	3756 3762	I
A10	3801-3869	ı	ı	3812 3839	3812 3839	3837 3855	3856 3862	ř ·
A14	3901-3969	1	ı	3939	3939	3937 3955	3956 3962	ı

THIS PAGE BLANK (USPTO)

The girth of the man of the court of the cou

Table 302 RERS sites found in Human Kappa FR1, continued

	BsaJI	BssKI (NstNI)	BpmI	BSLFI	Haell	Tsp5091
	xx29 xx42 xx43	xx22 xx30 xx43	xx20 xx41 xx44	Cac8I	I	
	-		>	NaeI		
-				NgoMI		
				۸		
VKI						
012 1-69		1	1	1	ı	1
02 101-169	1	1	1	-	_	-
018 201-269	-	-	1	ı	ı	ŀ
08 301-369	_	1		_	-	•
A20 401-469	-	_	-	_	-	ł
A30 501-569		-	-	1	1	-
L14 601-669	_	_	-	_	-	-
L1 701-769	_	-	1	1	_	_
L15 801-869	-	-	ı	_	_	_
14 901-969	_	-	_	-	1	1
L18 1001-1069	_		-	1	1	_
L5 1101-1169	_	_	_	1	-	_
L19 1201-1269	_	_	-	ı	_	_
L8 1301-1369	_	+	-	_	_	_
L23 1401-1469	_	_	I	ı	_	-
L9 1501-1569	1	-		1	-	1
L24 1601-1669	1	-	_	ı	-	1

of the first street in the fig. and the first street of the second street of the first street of the first

		BsaJI	BssKi (NstNI)	BpmI	BSEFI	Haell	Tsp509I
		xx29 xx42 xx43	xx22 xx30 xx43	xx20 xx41 xx44	Cac8I	н	
				>	NaeI		
					NgoMI		
					V		
L11	1701-1769	-	-	_	-	_	-
112	1801-1869	_	_	_	-	-	_
VKII							
011	1901-1969	. 1942	1943	1944	1981	1954	_
01	2001-2069	2042	2043	2044	2051	2054	1
A17	2101-2169	2142	1	-	2151	2154	_
A 1	2201-2269	2242	_	_	2251	2254	_
A18	2301-2369	2342	2343	_	2351	2354	_
A 2	2401-2469	2442	2443	_	2451	2454	I
A19	2501-2569	2542	2543	2544	2551	2554	-
A3	2601-2669	2642	2643	2644	2651	2654	-
A23	2701-2769	2742	_	_	2751	2754	-
VKIII							
A27	2801-2869	2843	2822 2843	2820 2841	-	-	2803
A11	2901-2969	2943	2943	2920 2941	ı	ı	2903
L2	3001-3069	3043	3043	3041	-	-	_
L16	3101-3169	3143	3143	3120 3141	_	1	1
16	3201-3269	3243	3243	3220 3241	ı	ı	3203
L20	3301-3369	3343	3343	3320 3341	1	I	3303

The first tent made of made than 11 than 11 than 11 cents of organization of the state of the st

		BsaJI	BssKI (NstNI)	BpmI	BSrFI	Haell	Tsp509I
		xx29 xx42 xx43	xx22 xx30 xx43	xx20 xx41 xx44	Cac8I	I	
				>	NaeI		
					NgoMI		
		į			^		
1.2	L25 3401-3469	3443	3443	3420 3441	1	ı	3403
. **	VKIV						
B3	3501-3569	3529	3530	3520	ı	3554	
VKV	W						
5 B2	3601-3669		3643	3620 3641	ı	-	
\$	VKVI						
AŽ	A26 3701-3769		_	3720	_	_	3703
A1	A10 3801-3869		1	3820	_	_	3803
A14	14 3901-3969	3943	3943	3920 3941	1	1	1

Table 400 Lambda FR1 GLG sequences

	,	VL1					•					
	•	ATT	CNG	ጥርጥ	GTG	CTC	እ ር ሞ	CAG	CCA	ccc	ጥርር	GTG TCT GAA
				CCC								
5												gtg tct gGG
,			_			_		_				tgC ! le
												gCg tct gGG
			_			_		_				tgt ! 1c
						-		-				gCg tct gGG
10			_			_		_				tgt ! 1g
10												gtg tct gCG
			_			_		_				tgC ! 1b
	1	VL2	gcc	CCA	GGA	cag	ang	gcc	acc	acc		cyc : 15
	•	V112	CAG	ጥርጥ	GCC	ርሞር	аст	CAG	ССТ	CCC	тсс	GCG TCC GGG
15												TGC ! 2c
10												gTg tcc ggg
			-		_	_		_				tgc! 2e
												gTg tcT ggg
												tgc ! 2a2
20						_						gTg tcc ggg
			_		_	_		_				tgc ! 2d
												gTg tcT ggg
			_		_	_		_				tgc ! 2b2
	!	VL3			J J	· J						3
25			TCC	TAT	GAG	CTG	ACT	CAG	CCA	CCC	TCA	GTG TCC GTG
			TCC	CCA	GGA	CAG	ACA	GCC	AGC	ATC	ACC	TGC! 3r
			tcc	tat	gag	ctg	act	cag	cca	cTc	tca	gtg tcA gtg
			Gcc	cTG	gga	cag	acG	gcc	agG	atT	acc	tgT ! 3j
			tcc	tat	gag	ctg	acA	cag	cca	ccc	tcG	gtg tcA gtg
30			tcc	cca	gga	caA	acG	gcc	agG	atc	acc	tgc! 3p
			tcc	tat	gag	ctg	acA	cag	cca	ccc	tcG	gtg tcA gtg
			tcc	сТа	gga	cag	aTG	gcc	agG	atc	acc	tgc ! 3a
			tcT	tCt	gag	ctg	act	cag	GAC	ccT	GcT	gtg tcT gtg

•

Gcc TTG gga cag aca gTc agG atc acA tgc ! 31

				tcc	tat	αΤα	ctq	act	cag	cca	ccc	tca	ata	tcF	A gtg
							_		_				tgT		3h
							-		-	_			_		\ gtg
							_		_				tgc		3e
	5						_		_	_			_		d gtg
						-	_		_				tgc		3m
															\ gtg
				tcT	ccĠ	gga	cag	aca	gcc	agG	atc	acc	tgc	!	V2-19
		!	VL4												
	10			CTG	CCT	GTG	CTG	ACT	CAG	ccc	CCG	TCT	GCA	TCI	GCC
				TTG	CTG	GGA	GCC	TCG	ATC	AAG	CTC	ACC	TGC	!	4c
				cAg	cct	gtg	ctg	act	caA	TcA	TcC	tct	gcC	tct	gcT
				tcc	ctg	gga	Tcc	tcg	Gtc	aag	ctc	acc	tgc	!	4a
				cAg	cTt	gtg	ctg	act	caA	TcG	ccC	tct	gcC	tct	gcc
₽₩,	15			tcc	ctg	gga	gcc	tcg	Gtc	aag	ctc	acc	tgc	!	4b
And And		!	VL5												
Į.				CAG	CCT	GTG	CTG	ACT	CAG	CCA	CCT	TCC	TCC	TCC	GCA
the of the trip				TCT	CCT	GGA	GAA	TCC	GCC	AGA	CTC	ACC	TGC	!	5e
IJ				cag	Gct	gtg	ctg	act	cag	ccG	Gct	tcc	CTc	tcT	'gca
Ta the	20			tct	cct	gga	gCa	tcA	gcc	agT	ctc	acc	tgc	!	5c
9				cag	cct	gtg	ctg	act	cag	cca	Tct	tcc	CAT	tcT	gca
				tct	Tct	gga	gCa	tcA	gTc	aga	ctc	acc	tgc	!	5b
14		!	VL6												
4.4				AAT	TTT	ATG	CTG	ACT	CAG	CCC	CAC	TCT	GTG	TCG	GAG
	25			TCT	CCG	GGG	AAG	ACG	GTA	ACC	ATC	TCC	TGC	!	6a
•		!	VL7												
				CAG	ACT	GTG	GTG	ACT	CAG	GAG	CCC	TCA	CTG	ACT	GTG
				TCC	CCA	GGA	GGG	ACA	GTC	ACT	CTC	ACC	TGT	!	7a
				cag	Gct	gtg	gtg	act	cag	gag	ccc	tca	ctg	act	gtg
	30			tcc	cca	gga	ggg	aca	gtc	act	ctc	acc	tgt	!	7b
		!	AF8												
															GTG
				TCC	CCT	GGA	GGG	ACA	GTC	ACA	CTC	ACT	TGT	!	8a

! VL9

CAG CCT GTG CTG ACT CAG CCA CCT TCT GCA TCA GCC

TCC CTG GGA GCC TCG GTC ACA CTC ACC TGC ! 9a

! VL10

5 CAG GCA GGG CTG ACT CAG CCA CCC TCG GTG TCC AAG

GGC TTG AGA CAG ACC GCC ACA CTC ACC TGC ! 10a

Table 405 RERSs found in human lambda FR1 GLGs ! There are 31 lambda GLGs MlyI NnnnnnGACTC 25

6: 7: 6 1: 3: 6 6 8: 6 4: 6 5 9: 6 10: 6 11: 6 12: 6 15: 6 16: 6 20: 21: 22: 6 23: 23: 50 24: 25: 25: 50 27: 6 28: 6 30: 6 . 6 26: 6

31: 6

There are 23 hits at base# 6

10

Hard short there we three deals had the

(J

-"- GAGTCNNNNNn 1

26: 34

15 1: 2: 3: 9 4: 9 11: 9 11: 56 12: 13: 9 14: 9 16: 9 17: 9 18: 9 24: 9 9 26: 9 19: 9 20: 9 23: 9 25: 30: 31: 9

There are 19 hits at base# 9

20 HinfI Gantc 27

1: 12 3: 12 4: 12 6: 12 7: 12 8: 12 9: 12 10: 12 12: 12 15: 12 11: 12 16: 12 22: 12 20: 12 21: 12 23: 12 23: 46 23: 56 24: 12 25: 12 25: 56 26: 12 26: 34 27: 12

25 28: 12 30: 12 31: 12

There are 23 hits at base# 12

PleI gactc 25

30 20: 12 21: 12 22: 12 23: 12 23: 56 24: 12

31: 12

There are 23 hits at base# 12

26: 34

```
1: 14
                   2: 24
                             3: 14
                                       3: 24
                                                 4: 14
                                                           4: 24
         5: 24
                   6: 14
                             7: 14
                                      7: 24
                                                 8: 14
                                                           9: 14
    5
        10: 14
                  11: 14
                            11: 24
                                      12: 14
                                                12: 24
                                                          15:
                                                               5
        15: 14
                  16: 14
                            16: 24
                                      19: 24
                                                20: 14
                                                          23: 14
                  25: 14
        24: 14
                            26: 14
                                      27: 14
                                                28: 14
                                                          29: 30 .
        30: 14
                  31: 14
        There are 21 hits at base# 14
   10
       BsaJI Ccnnqq
                                         38
         1: 23
                   1: 40
                           2: 39
                                       2: 40
                                                 3: 39
                                                          3: 40
         4: 39
                  4: 40
                            5: 39
                                      11: 39
                                                12: 38
                                                          12: 39
        13: 23
                 13: 39
                            14: 23
                                      14: 39
                                               15: 38
                                                         16: 39
   15
        17: 23
                 17: 39
                           18: 23
                                      18: 39
                                               21: 38
                                                         21: 39
And the first the first the first that the
        21: 47
                 22: 38
                            22: 39
                                      22: 47
                                               26: 40
                                                          27: 39
        28: 39
                  29: 14
                            29: 39
                                      30: 38
                                               30: 39
                                                          30: 47
        31: 23
                  31: 32
        There are 17 hits at base# 39
   20
        There are
                   5 hits at base# 38
0.01
        There are
                     5 hits at base# 40 Makes cleavage ragged.
      MnlI cctc
                                         35
         1: 23
                   2: 23
                             3: 23
                                       4: 23
                                                5: 23
                                                           6: 19
14
7, 7
         6: 23
                  7: 19
                            8: 23
                                       9: 19
                                                9: 23
                                                         10: 23
   25
        11: 23
                 13: 23
                           14: 23
                                      16: 23
                                                         18: 23
                                               17: 23
        19: 23
                 20: 47
                           21: 23
                                      21: 29
                                               21: 47
                                                         22: 23
       22: 29
                 22: 35
                           22: 47
                                      23: 26
                                               23: 29
                                                         24: 27
       27: 23
                 28: 23
                           30: 35
                                      30: 47
                                               31: 23
       There are 21 hits at base# 23
   30
       There are
                   3 hits at base# 19
       There are
                    3 hits at base# 29
       There are
                    1 hits at base# 26
                     1 hits at base# 27 These could make cleavage ragged.
       There are
            gagg
                                          7
   35
        1: 48
                  2: 48
                            3: 48
                                      4: 48
                                               27: 44
                                                         28: 44
```

32

DdeI Ctnag

29: 44

£. 4.

[]

=== ļŁ

7.

O

```
BssKI Nccngg
                                        39
        1: 40
                  2: 39
                            3: 39
                                     3: 40
                                               4: 39
                                                         4: 40
    5
        5: 39
                  6: 31
                            6: 39
                                     7: 31
                                               7: 39
                                                         8: 39
        9: 31
                  9: 39
                           10: 39
                                    11: 39
                                              12: 38
                                                        12: 52
       13: 39
                 13: 52
                           14: 52
                                    16: 39
                                              16: 52
                                                        17: 39 .
       17: 52
                 18: 39
                           18: 52
                                    19: 39
                                              19: 52
                                                        21: 38
       22: 38
                                    26: 39
                 23: 39
                           24: 39
                                              27: 39
                                                        28: 39
   10
       29: 14
                 29: 39
                           30: 38
       There are 21 hits at base# 39
       There are
                    4 hits at base# 38
       There are
                    3 hits at base# 31
       There are
                    3 hits at base# 40 Ragged
  15
      BstNI CCwgg
                                       30
                  2: 40
        1: 41
                           5: 40
                                     6: 40
                                               7: 40
                                                         8: 40
        9: 40
                 10: 40
                          11: 40
                                    12: 39
                                              12: 53
                                                       13: 40
13
13
20
       13: 53
                 14: 53
                           16: 40
                                    16: 53
                                              17: 40
                                                       17: 53
       18: 40
                 18: 53
                           19: 53
                                    21: 39
                                              22: 39
                                                       23: 40
       24: 40
                 27: 40
                           28: 40
                                    29: 15
                                              29: 40
                                                       30: 39
       There are 17 hits at base# 40
                   7 hits at base# 53
       There are
                    4 hits at base# 39
       There are
$≜ 25
       There are
                    1 hits at base# 41 Ragged
      PspGI ccwqq
                                       30
                  2: 40
        1: 41
                           5: 40
                                     6: 40
                                               7: 40
                                                        8: 40
        9: 40
                 10: 40
                                    12: 39
                          11: 40
                                              12: 53
                                                       13: 40
  30
       13: 53
                 14: 53
                                    16: 53
                          16: 40
                                              17: 40
                                                       17: 53
       18: 40
                 18: 53
                          19: 53
                                    21: 39
                                              22: 39
                                                       23: 40
       24: 40
                 27: 40
                          28: 40
                                    29: 15
                                              29: 40
                                                       30: 39
       There are 17 hits at base# 40
       There are
                    7 hits at base# 53
  35
       There are
                  4 hits at base# 39
```

There are 1 hits at base# 41

		ScrF	I CC1	ngg				;	39				
		1:	41	2:	40	3:	40	3:	41	4:	40	4:	41
	5	5:	40	6:	32	6:	40	7:	32	7:	40	8:	40
		9:	32	9:	40	10:	40	11:	40	12:	39	12:	53
		13:	40	13:	53	14:	53	16:	40	16:	53	17:	40
		17:	53	18:	40	18:	53	19:	40	19:	53	21:	39
		22:	39	23:	40	24:	40	26:	40	27:	40	28:	40
	10	29:	15	29:	40	30:	39						
		The	re an	ce 2	l hi	ts at	bas	e# 40					
		The	re aı	ce 4	4 hi	ts at	bas	e# 39					
		The	re a	e :	3 hi	ts at	bas	e# 41					
r.	15	MaeI	II gt	nac				:	16				
of their spirit spirit.		1:	52	2:	52	3:	52	4:	52	5:	52	6:	52
13		7:	52	9:	52	26:	52	27:	10	27:	52	28:	10
		28:	52	29:	10	29:	52	30:	52				
(3		The	re an	re 13	3 hi	ts at	bas	e# 52					
	20		_										
2.7a		Tsp4	•						15				
23 223			52		52		52		52		52	6:	
14			52		52			27:	52	28:	10	28:	52
(J	25	29:		29:		30:		" 50					
14	25	The	re ar	re 12	2 hi	ts at	bas	e# 52					
		HphI	tasa					,	26				
		1:		2:	53	3:	52		53	5 :	52	6.	53
						9:							
	30			17:									
	50		59			24:							
			59			21.	55	25.	33	27.	55	20.	3,5
						ts at	bas	e# 59					
						ts at							
	35			,		.=							

BspMI ACCTGCNNNNn

11: 61 13: 61 14: 61 17: 61 18: 61 19: 61

14

20: 61 21: 61 22: 61 23: 61 24: 61 25: 61

30: 61 31: 61

5 There are 14 hits at base# 61 Goes into CDR1

~ ~...

Table 500: h3401-h2 captured Via CJ with BsmAI 2 1 3 4 5 6 7 8 9 10 11 12 13 14 15 Ι T S Ρ T D Q М 0 Α aGT GCA Caa gac atc cag atg acc cag tct cca gcc acc ctg tct 5 ! ApaLI... a gcc acc! L25, L6, L20, L2, L16, A11 ! Extender..... 21 26 20 22 23 24 25 27 -28 29 30 ! 16 17 18 19 10 ! V G T L S S Ρ Ε R Α C R gtg tct cca ggg gaa agg gcc acc ctc tcc tgc agg gcc agt cag 33 36 37 38 40 ! 31 32 34 35 39 41 42 43 44 L S V S N N Α W Y Q Q K Ρ G 0 15 agt gtt agt aac aac tta gcc tgg tac cag cag aaa cct ggc cag 52 56 57 ! 46 47 48 49 50 51 53 54 55 58 59 60 ! V R L \mathbf{L} Ι Y G Α S Т R Α D gtt ccc agg ctc ctc atc tat ggt gca tcc acc agg gcc act gat 20 ! 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 Ρ Α R \mathbf{F} S G S G S G T D F atc cca gcc agg ttc agt ggc agt ggg tct ggg aca gac ttc act *25* ! 76 77 78 79 80 82 83 84 85 86 90 81 87 88 89 Τ S L Ε Ρ E F Α V Y Y Ι R D ctc acc atc agc aga ctg gag cct gaa gat ttt gca gtg tat tac 98 ! 91 92 93 94 95 96 97 99 100 101 102 103 104 105 S Q R Y G S P G W Τ F Q G tgt cag cgg tat ggt agc tca ccg ggg tgg acg ttc ggc caa ggg ! 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 V Ε Ι K R \mathbf{T} V Α Α Ρ S 35 acc aag gtg gaa atc aaa cga act gtg gct gca cca tct gtc ttc ! 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 F Ρ Ρ S D Ε 0 $\mathbf L$ K S G atc ttc ccg cca tct gat gag cag ttg aaa tct gga act gcc tct 40 ! 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 ! V L ${f L}$ N N F Y Ρ R Ε V С Α K V gtt gtg tgc ctg ctg aat aac ttc tat ccc aga gag gcc aaa gta

```
K
                      V
                          D
                              N
                                  Α
                                       L
                                           Q
                                               S
                                                   G
                                                        N
        cag tgg aag gtg gat aac gcc ctc caa tcg ggt aac tcc cag gag
   5 ! 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
        S
             V
                 Т
                     E
                          Q
                              D
                                  S
                                      K
                                           D
                                               S
                                                   T
                                                        Y
                                                            S
                                                                L
        agt gtc aca gag cag gac agc aag gac agc acc tac agc ctc agc
      ! 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
   10 ! S
             T
                      Т
                 L
                          L
                              S
                                               Y
                                  K
                                       Α
                                           D
                                                   E
                                                        K
                                                            Η
                                                                K
                                                                    V
        agc acc ctg acg ctg agc aaa gca gac tac gag aaa cac aaa gtc
      ! 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
      ! Y
             Α
                С
                     \mathbf{E}
                          V
                              Т
                                  Η
                                      Q
                                          G
                                              L
                                                   S
                                                      S
                                                           Ρ
                                                                V
   15
        tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg cct gtc aca
      ! 211 212 213 214 215 216 217 218 219 220 221 222 223
                 \mathbf{F}
                     N
                         K
                             G
                                  Ε
                                     С
                                           K
                                               G
                                                   E
        aag agc ttc aac aaa gga gag tgt aag ggc gaa ttc gc.....
. .
ť0
Table 501: h3401-d8 KAPPA captured with CJ and BsmAI
             2
                 3
                      4
                          5
                              6
                                  7
                                       8
                                           9
                                              10
                                                  11
                                                       12
                                                           13
                                                                   15
[]
  25
      ! S
             Α
                 Q
                     D
                          Ι
                              0
                                  Μ
                                       Τ
                                           Q
                                               S
                                                   Ρ
                                                       Α
                                                            T
                                                                L
                                                                    S
       aGT GCA Caa gac atc cag atg acc cag tct cct gcc acc ctg tct
        ApaLI...Extender......<u>a</u> gcc acc !
The 11 11.41
      L25,L6,L20,L2,L16,A11
                                                    A GCC ACC CTG TCT ! L2
  30
٠. ا
         16
             17
                 18
                      19
                          20
                              21
                                  22
                                      23
                                           24
                                               25
                                                   26
                                                       27
                                                            28
                                                                29
Ü
                                                                    30
                 Ρ
                     G
                          Ε
                              R
                                  Α
                                       Т
                                           L
                                               S
                                                   С
                                                       R
                                                            Α
                                                                S
                                                                    0
        gtg tct cca ggt gaa aga gcc acc ctc tcc tgc agg gcc agt cag
      ! GTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC
                                                       !
  35
         31
             32
                 33
                     34
                          35
                              36
                                  37
                                       38
                                           39
                                              40
                                                   41
                                                        42
                                                            43 44
                                                                    45
        N
                 L
                      S
                          N
             L
                              L
                                  Α
                                       W
                                           Y
                                               Q
                                                   Q
                                                       K
                                                            Ρ
        aat ctt ctc agc aac tta gcc tgg tac cag cag aaa cct ggc cag
  40 ! 46
             47
                 48
                     49
                          50
                                  52
                              51
                                       53
                                           54
                                               55
                                                   56
                                                       57
                                                            58
                                                                59
                                                                    60
         Α
                                  Y
                 R
                     L
                          L
                              Ι
                                      G
                                           Α
                                               S
                                                   Т
                                                       G
                                                                Τ
                                                            Α
                                                                    G
        gct ccc agg ctc ctc atc tat ggt gct tcc acc ggg gcc att ggt
        61
             62
                 63
                     64
                          65
                              66
                                 67
                                       68
                                           69
                                               70
                                                   71
                                                       72
                                                            73
                                                                74
                                                                    75
  45
     ! I
             Ρ
                 Α
                     R
                          F
                              S
                                  G
                                       S
                                           G
                                               S
                                                   G
                                                       Τ
                                                            E
                                                                F
```

! 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165

. .

atc cca gcc agg ttc agt ggc agt ggg tct ggg aca gag ttc act

```
! 76
           77
              78
                  79 80 81
                            82 83 84 85 86 87
                                                   88
                                           F
     ! L
           Т
               Ι
                  S
                      S
                          L
                             0
                                S
                                     Ε
                                        D
                                               Α
                                                   V
                                                       Y
                                                           F
       ctc acc atc agc agc ctg cag tct gaa gat ttt gca gtg tat ttc
           92
               93
                   94
                      95
                         96 97
                                98
                                     99 100 101 102 103 104 105
     ! C
                  Y
           Q
               Q
                      G
                          T
                             S
                                 Ρ
                                     Ρ
                                        Т
                                           F
                                               G
                                                   G
                                                       G
       tgt cag cag tat ggt acc tca ccg ccc act ttc ggc gga ggg acc
     ! 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
  10 ! K
           V
              E
                     K
                         R
                             T V A
                 Ι
                                       Α
                                           Ρ
                                               S
                                                   V
       aag gtg gag atc aaa cga act gtg gct gca cca tct gtc ttc atc
     ! 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
     ! F P P S D E Q L K S G T A
                                                       S V
      ttc ccg cca tct gat gag cag ttg aaa tct gga act gcc tct gtt
     ! 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150
     ! V C P L
                     N
                            F Y P R
                         N
                                           E
                                               Α
                                                  K
                                                      V
       gtg tgc ccg ctg aat aac ttc tat ccc aga gag gcc aaa gta cag
  20
     ! 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165
              V D
                     N
                        A L Q S G
                                          N
                                              S
                                                   Q
                                                      Ε
O
       tgg aag gtg gat aac gcc ctc caa tcg ggt aac tcc cag gag agt
U
  25 ! 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
     ! V T
              E Q
                     D
                         N
                            K
                                D
                                   S
                                       T
                                           Y
                                               S
                                                  L
                                                       S
O
       gtc aca gag cag gac aac aag gac agc acc tac agc ctc agc agc
Įħ.
     ! 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
  30 ! T
               Т
                     S
                         K
                            V D
                                   Y
           L
                  L
                                        \mathbf{E}
                                            K
                                               Η
                                                   Ε
                                                       V
       acc ctg acg ctg agc aaa gta gac tac gag aaa cac gaa gtc tac
14
4
     ! 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
IJ
     ! A C E V T H Q G L S S P V
  35
      gcc tgc gaa gtc acc cat cag ggc ctt agc tcg ccc gtc acg aag
     ! 211 212 213 214 215 216 217 218 219 220 221 222 223
          F
             N R G E C K K E
                                          F
                                              V
       agc ttc aac agg gga gag tgt aag aaa gaa ttc gtt t
  40
```

The first construction of the street and the construction of the street of the street

Table 508 Human heavy chains bases 88.1 to 94.2

Number of sequences..... 840

			1										
5			Nur	Number	of N	Mismat		chers.	:	:		Probe	
	Id	Ntot	0	1	2	3	4	5	9	7	Name	Sequence	Dot form
		364	152	97	16	26	7	4	2	0	VHS881-1.1	gctgtgtattactgtgcgag	gctgtgtattactgtgcgag
	7	265	150	09	33	13	Ŋ	4	0	0	VHS881-1.2	gccgtgtattactgtgcgag	
	ო	96	14	34	16	10		7	თ	1	VHS881-2.1	gccgtatattactgtgcgag	a
01	4	20	0	က	4	თ	2	2	0	0	VHS881-4.1	gccgtgtattactgtacgag	aa
	2	.95	25	36	18	11	2	2	٥	1	VHS881-9.1	gccatgtattactgtgcgag	ca
		840	341	230	147	69	21	19	11	2			
			341	571	718	787	808	827	838	840			
15				88	89 90	91	92	93 94	4 95		Codon number as i	in Table 195	
				Reco	Recognition	:ion	•	•	:	Sten	Stem Loop.	Stem	
	(VHS881	31-1.1	2	'-gctgtgtat tac	ıtgta	t t	act-	t-gtgcgag	gag	CAC.		cAcggATgTg-3'	
	(VHS881	31-1.2)		စ်သည်.	gccgtgtat tac	<u>t</u>	act-	t-gtgcgag	gag	CAC.			
20	B8SHV)	VHS881-4.1)	5 1	500B	gccgtatat tac gccgtgtat tac	<u>i</u>	act-	gccgtatat tact-gtgcgag gccgtgtat tact-gt a cgag	gag		cacaleegig 11911 cacaleegig Tigit	cac <u>ggarg</u> rg-3 cacggargrg-3'	
	(VHS8	(VHS881-9.1)	-15	5'-gccatgtat	tate	tt	tact-	t-gtgcgag site o	gag e of	S n	U	cAc<u>ggATg</u>Tg- 3' e	
	(FOKJact)	(act)	51-	5'-c <u>AcAtcc</u> gTg	TCC		TTgT	gTT cA	c <u>gg</u> A	cacggatgTg-3	-3,		
25													
	(VHEx881)		5'-AATAGTAGAc TGCAGTGTcc AGAGTATCT TAGAGTATCT	AATAGTAGAC TGCAGTGTCC AGAGTATTCT TAGAGTTGTC	AgAc TCT	Tgc	AgTg		TCAG	TCTAGCCCTTA	AgcTgTTcAT gTgAAgcg-3'	cTgcAAgTAg-	
	! note	that VHEx881 is the reverse	VHEX	:881	is t	he:	reve		Comp	complement	of the	ON below	
1		=	[RC] 5	5'-cgCttcacTaag	Cttc	acT	aag-		I I				
30				SS	Scab	:	:						
				Sy	Synthetic	tic	3-23		in	as in Table	206		
				_	'CT F	\GA	gac	aac	tct	aagle	at act ctc ta	TCT AGA gac aac tct aag aat act ctc tac ttg cag atg -	
	- .			×	XbaI	:							
3,6				<u>~</u>	ac e	l De l	TTA	AGg	gct	gaglç	aac agC TTA AGg gct gag gac aCT GCA Gtc tac tat t-3	c tac tat t-3'	
\mathcal{C}						Αŧ	Aflii.	:					
	(VHBA881)	181)	5	_	-cgCttcacTaag	acT	aag-						
					CT Z	\GA	gac	aac	tct	aagle	at act ctc ta	TCT AGA gac aac tct aag aat act ctc tac ttg cag atg -	
					ac a	1gc	TTA	AGg	gctl	gaglç	aac agC TTA AGg gct gag gac aCT GCA Gtc tac tat tgt	c tac tat tgt gcg ag-3'	_
	(VHBB881	381)	S	5'-cq	-cqCttcacTaaq	acT	aad						

The first Cip with right regs that then the thirt it into a sum that when the first off with with the first off the first off with the first off the first o

|TCT|AGA|qac|aac|tct|aaq|aat|act|ctc|tac|ttq|caq|atq|-

TCT A6A gac aac tct aag aat act ctc tac ttg cag atg - aac agC TTA AGg gct gag gac aCT GCA Gtc tac tat tgt Acg ag-3'				Sequence Dot Form	gacccagtctccatcctcc gacccagtctccatcctcc	gactcagtctccactctcctct	gacgcagtctccaggcaccggg.a	gacgcagtctccagccaccggga		
aat act ct gac aCT GC	-3'			Name S	SK12012 g	SK12A17 g	SK12A27 g	SK12A11 g		
l aag I gag	aac			9	0	⊣	0	0	Ļ	82
: tct gct	l gac	•		5	0	0	0	0	0	7 175 178 181 181 182
: Jaac NAGG	l AG			4	7	-	0	0	ო	181
N gac	I TCI	2-30		Э	1	7	0	0	ო	178]
r AG c ag(Taac	ases 12-30		1 2 3 4	20	9	Н	7	28	175
	ttca	base	-	1	21	က	æ	18	20	
	cgc	ppa,		0	40 2	19	17	21	16	97 14
	(VH881PCR) 5'-cgCttcacTaag TCT AGA gac aac -3'	5 Table 512: Kappa, b		ID Ntot 0	84	32	56	40	182	
	н881]	ble		ID	1	7	ო	4		
	2	Та								
		5					10			

FokI.

FokI.

The first offer the Hallen of the first offer of the other offer o

What happens in the upper strand:

5'-gac cca gtc tcc a-tc ctc c-3' Site of cleavage in substrate	, 5'-gac tca gtc tcc a-ct ctc c-3'	5'-gac gca gtc tcc a-gg cac c-3'	5'-gac gca gtc tcc a-gc cac c-3'	5'-ccTctactctTgTcAcAgTgcAcAA gAc ATc cAg-3' !sense strand ScabApaLI.	(kapextUREPCR) 5'-ccTctactctTgTcAcA <u>gTg</u> -3' Scab	5'-ggAggATggA cTggATgTcT TgTgcAcTgT gAcAAgAgTA gAgg-3' 5'-ccTctactctTgTcAcA <u>gTgcAc</u> AA gAc ATc cAg tcc a-tc ctc c-3' ON above 1s R.C. of th1s one	5 -ggAgAgIggA cIggAIgIci igigcAcigi gAcAAgAgiA gAgy-3 5'-ccTctactctTgTcAcA <u>gTgcAc</u> AA gAc ATc cAg tcc a-ct ctc c-3' ON above is R.C. of this one 5' comment and management of this one	5 -ggigceigga eiggaigiei igigeacigi gacaagagia gagg-5 5'-ccTetaetetTgTeAcA <u>gTgcAc</u> AA gAc ATc cAg tec a-gg cac c-3' ON above 1s R.C. of this one 5'-cmmman amaganaman mamaanaman anganaman anganaman anga cac c-3' ON above 1s R.C. of this one	s -ggiggcigga ciggaigicí igigcacigí gacaagagas gagg-s 5'-ccTctactctTgTcAcA <u>gTgcAc</u> AA gAc ATc cAg tcc a-gc cac c-3' ON above 1s R.C. of this one ScabApaLI.
(SzKB1230-012*)	: (SzKB1230-A17*)	(SzKB1230-A27*)	: (SzKB1230-A11*)	(kapextURE)	(kapextUREPCR)	[]	C]	(၂	(Kabro40K) 5

The thirty of the the thirty that the thirty then the thirty that the thirty t

|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-

The first case than the major case for the first case of the first

	5'-gac cca gtc tcc a-tc ctc c-3' Site of cleavage in substrate	5'-gac tca gtc tcc a-ct ctc c-3'	5'-gac gca gtc tcc a-gg cac c-3'	5'-gac gca gtc tcc a-gc cac c-3'	5'-ccTctactctTgTcAcA <u>gTgcAc</u> AA gAc ATc cAg-3' !sense strand ScabApaLI.	TcAcA <u>gTg</u> -3'	5'-ggAggATggA cTggATgTcT TgTgcAcTgT gAcAAgAgTA gAgg-3' 5'-ccTctactctTgTcAcA <u>gTgcAc</u> AA gAc ATc cAg tcc a-tc ctc c-3' ON above is R.C. of this one 5'-ggAgAgTggA cTggATgTcT TgTgcAcTgT gAcAAgAgTA gAgg-3' 5'-ccTctactctTgTcAcA <u>gTgcAc</u> AA gAc ATc cAg tcc a-ct ctc c-3' ON above is R.C. of this one	5'-ggTgccTggA cTggATgTcT TgTgcAcTgT gAcAAgAgTA gAgg-3' 5'-ccTctactctTgTcAcA <u>RTgcAc</u> AA gAc ATc cAg tcc a-gg cac c-3' ON above 1s R.C. of th1s one 5'-ccTctactctTgTcAcA <u>RTgcAc</u> AA gAc ATc cAg tcc a-gg cac c-3' ON above 1s R.C. of th1s one 5'-ccTctactctTgTcAcA <u>RTgcAc</u> AA gAc ATc cAg tcc a-gc cac c-3' ON above 1s R.C. of th1s one ScabApaLI.
What happens in the upper strand:	(SzKB1230-012*) 5'-gac ;	(SzKB1230-A17*) 5'-gac	: (SzKB1230-A27*) 5'-gac	10 (SzKB1230-A11*) 5'-gac	(kapextURE) 5'-ccTctactctTgTcAcAgTgcAc	<pre>// Is (lapextUREPCR) 5'-ccTctactctTgTcAcAgTg-3'</pre>	C C	<pre>(kaBRO3UR) 5'-ggTgccTggA cTggATgTcT TgTgcAc ! [RC] 5'-ccTctactctTgTcAcAgTgcAcA. (kaBRO4UR) 5'-ggTggcTggA cTggATgTcT TgTgcAc 25 ! [RC] 5'-ccTctactctTgTcAcAgTgcAcA.</pre>

19.3		
.3 to	128	
13		
bases 13.3 to 19.3	sequences	
LS	•	
pte	:	
adapters	ŝ	
H	nce	
5	Ine	
Fable 515 Lambda URE	sec	
Cam	umber of	
15 I	er	
e 5	dil	
apl	ž	
H	 	-

	Dot form	gtctcctggacagtcgatc	.g.cttga.ag	ag	.g.cagag.g	•														
	Dot f	gtctc	.g.ct		.g.c.	1														
	Sequence	gtctcctggacagtcgatc	ggccttgggacagacagtc	gtctcctggacagtcagtc	ggccccagggcagagggtc			•	.ggAgAc-3' gaar gra-3'	, n- m		AAggcc-3'	<u>qATq</u> Tg-3' !		• • • • • • • • • • • • • • • • • • • •	.ggAgAc-3'	qATqTg-3	•		ggggcc-3' qATqTq -3'
	Name	VL133-2a2	VL133-31	VL133-2c	VL133-1c			cognition	5'-cAcAlcogIg TIgII cAcggAIgIg gAIcgAcIgIccAggAgAc-3 5'-atctectagacagtcate cAcAlcogIg AAcAA cacaaagaaa-3	Recognition Stem Loop. Stem	Recognition	5'-cAcATccgTg TTgTT cAcggATgTg gAcTgTcTgTcccAAggcc-3	<pre>[RC] 5'-ggccttgggacagacagtc cAcAIccgTg AAcAA cAcgAIgTg-3 Recognition Stem Loop. Stem</pre>	•	loop. Stem Recognition	Ig TIGIT cAcggATgTg gAcTgAcTgTccAggAgAc-3	5'-gtctcctggacagtcagtc cAcArccgrg AAcAA cAcggArgrg-3	Recognition Stem Loop. Stem	Recognition	5'-cAcATccgTg TTgTT cAcggATgTg gAcccTcTgcccTggggcc-3' [RC] 5'-ggccccagggcagagggtc cAcATccgTg AAcAA cAcggATgTg -3'
	- ω	П	7	0	2	2	128	. Re	Tg g ^p	" : ∭ :	Re	Tg gA	<u>eegT</u> 		: Re	Tg g⊉	<u>ccg</u> 1g	:	. Re	Tg g ^g ccgTg
	7	7	0	2	4	11	123		ggATg	stem.	loop. Stem	gATg	Stem.		n	1gATg	ACAT	stem.	loop. Stem	gaatg
	9	2	Н	٦	7	11	112	Ster	cAcc		Ster	cAcc	gtc		Sten	c.Acc	gtc	:	Sten	cAcq gtc 6
les	5	0) 1	1	1 3	8 5	96 101	oop.	TTgTT	,	000	TgT	ıgaca		.doo	TgT	ıgtca		oop.	TgTT
match	3 4	0	1	0	4	5 8	88 96		gTg T	ggac. tion.		gTg	ggace tion.		:	gTg	ggace	cion.		gTg T
mis	2	-	0	0	0	.1	83 8	Stem	ATCC	ogni	Stem	ATcc	cttg	1	Stem	ATcc	itact.	ogni	Stem	ATCC
Number of mismatches.		7	-	0	0	8	72 8	Ste	5'-cAcATccg' 5'-atctcctg	Rec	Ste	'-cAc	'-ggo Rec		Ste	5'-cAcATccg	'-gtc	Rec	Ste	'-cAc
qunN	0	45	10	9	3	64	64		5 [RC] 5			5	RC] 5				[RC] 5			5 RC] 5
 	Ntot	58	16	17	37	128				<u>.</u>										
	i Id	i 1	2	۳ 	4			. .,	(VL133-2a2)		- .	(VL133-31)				(VL133-2c)				(VL133-1c)
3	,	•			01				15			20			(22				30

```
What happens in the top strand:
!
(VI.133-2a2*) 5'-g tet eet gl
```

7,1

U

A. H.

ſħ

Œ

44

```
I site of cleavage in the upper strand
    (VL133-2a2*)
                   5'-g tct cct g|ga cag tcg atc
 5
    (VL133-31*)
                   5'-g gcc ttg g|ga cag aca gtc
    (VL133-2c*)
                   5'-g tct cct g|ga cag tca gtc
10
                   5'-g gcc cca g|gg cag agg gtc
   (VL133-1c*)
    ! The following Extenders and Bridges all encode the AA sequence of 2a2 for
    codons 1-15
15
    (ON LamEx133) 5'-ccTcTqAcTqAqT gcA cAq -
                         4
                              5
                                  6
                                      7
                                          8
                                               9
                                                   10
                                                        11
               AGt gcT TtA acC caA ccG gcT AGT gtT AGC ggT-
20
                13
                     14
                        15
               tcC ccG g !
                              2a2
    (ON LamB1-133) [RC] 5'-ccTcTqAcTqAqT gcA cAq -
25
                 2
                     3
                              5
                                  6
                                      7
                                               9
                                           8
                                                   10
                                                        11
               AGt gcT TtA acC caA ccG gcT AGT gtT AGC ggT-
                13
                     14
               tcC ccG q ga cag tcg at-3'!
                                               2a2 N.B. the actual seq is the
30
                                                     reverse complement of the
                                                     one shown.
    (ON_LamB2-133) [RC] 5'-ccTcTgAcTgAgT gcA cAg -
35
                 2
                              5
                     3
                                  6
                                      7
                                           8
                                               9
                                                   10
               AGt gcT TtA acC caA ccG gcT AGT gtT AGC ggT-
                13
                     14
                         15
               tcC ccG g ga cag aca gt-3' ! 31
                                                  N.B. the actual seq is the
40
                                                     reverse complement of the
                                                    one shown.
    (ON Lamb3-133) [RC] 5'-ccTcTqAcTqAgT gcA cAq -
45
                              5
                                  6
                                      7
                                           8
                                               9
                                                   10 11
               AGt gcT TtA acC caA ccG gcT AGT gtT AGC ggT-
                     14
                13
                        15
50
               tcC ccG g ga cag tca gt -3'! 2c N.B. the actual seq is the
                                                    reverse complement of the
                                                    one shown.
    (ON LamB4-133) [RC] 5'-ccTcTgAcTgAgT gcA cAg -
55
```

j

! 2 3 4 5 6 7 8 9 10 11 12
AGt gcT TtA acC caA ccG gcT AGT gtT AGC ggT!
! 13 14 15
5 tcC ccG g gg cag agg gt-3'! 1c N.B. the actual seq is the reverse complement of the one shown.
!
(ON_Lam133PCR) 5'-ccTcTgAcTgAgT gcA cAg AGt gc-3'

Table 525 ONs used in Capture of kappa light chains using CJ method and BsmAI

All ONs are written 5' to 3'.

gggAggATggAgAcTgggTc	gggAAgATggAgAcTgggTc	gggAgAgTggAgAcTgAgTc	gggTgccTggAgAcTgcgTc	gggTggcTggAgAcTgcgTc	gggAgTcTggAgAcTgggTc
5 REdapters (6) ON_2OSK15012	ON_20SK15L12	ON_20SK15A17	ON_20SK15A27	10 ON_2OSK15A11	ON_20SK15B3

Bridges (6)

BBBABBATBBABACTBBBTCATCTBBATBTCTTBTBCACTBTBACABABB BBBAABATBBABACTBBBTCATCTBBTBTCATTBTBACABABB BBBABABTBBACTBBBTCATCTBBACTTBTBCACTBTBACABABB BBBTBCCTBBABACTBBBTCATCTBBACTTBTBCACTBTBACABABB BBBTBBCTBBABACTBBBTCATCTBBACTTBTBCACTBTBACABABB BBBABTCTBBABACTBBBTCATCTBBATBTCTTBTBCACTBTBACABABB kapbr11012 kapbri1L12 kapbri1A17 kapbrilA27 kapbri1A11 kapbri1B3 15 20

Extender (5' biotinylated)

kapext1bio ccTcTgTcAcAgTgcAcAAgAcATccAgATgAcccAgTcTcc

Primers

25

kaPCRt1 ccTcTgTcAcAgTgcAcAAgAc sapfor 5'-aca ctc tcc cct gtt gaa gct ctt-3'

30 Table 530

PCR program for amplification of kappa DNA

95°C 5 minutes

95°C 15 seconds

65°C 30 seconds

1 minute 7 minutes hold 72°C 72°C 4°C

5

50 ng 1x Reagents (100 ul reaction):
Template
10x turbo PCR buffer
turbo Pfu

dNTPs kaPCRt1 kapfor

200 μM each 300 nM 300 nM

10

Real of the second of many that the thing that the test of the things of the test of the t

Table 610: Stuffer used in VH

	1	1 TCCGGAGCTT CAGATCTGTT TGCCTTTTTG TGGGGTGGTG CAGATCGCGT TACGGAGATC	CAGATCTGTT	TGCCTTTTTG	TGGGGTGGTG	CAGATCGCGT	TACGGAGATC
	61	61 GACCGACTGC TTGAGCAAAA GCCACGCTTA ACTGCTGATC AGGCATGGGA TGTTATTCGC	TTGAGCAAAA	GCCACGCTTA	ACTGCTGATC	AGGCATGGGA	TGTTATTCGC
	121	121 CAAACCAGTC GTCAGGATCT TAACCTGAGG CTTTTTTAC CTACTCTGCA AGCAGGGACA	GTCAGGATCT	TAACCTGAGG	CTTTTTTAC	CTACTCTGCA	AGCAGCGACA
5	181	181 TCTGGTTTGA CACAGAGCGA TCCGCGTCGT CAGTTGGTAG AAACATTAAC ACGTTGGGAT	CACAGAGCGA	TCCGCGTCGT	CAGTTGGTAG	AAACATTAAC	ACGTTGGGAT
	241	GGCATCAATT	GGCATCAATT TGCTTAATGA TGATGGTAAA ACCTGGCAGC AGCCAGGCTC TGCCATCCTG	TGATGGTAAA	ACCTGGCAGC	AGCCAGGCTC	TGCCATCCTG
	301	301 AACGTTTGGC TGACCAGTAT GTTGAAGCGT ACCGTAGTGG CTGCCGTACC TATGCCATTT	TGACCAGTAT	GTTGAAGCGT	ACCGTAGTGG	CTGCCGTACC	TATGCCATTT
	361	361 GATAAGTGGT ACAGCGCCAG TGGCTACGAA ACAACCCAGG ACGGCCCAAC TGGTTCGCTG	ACAGCGCCAG	TGGCTACGAA	ACAACCCAGG	ACGGCCCAAC	TGGTTCGCTG
	421	421 AATATAAGTG TTGGAGCAAA AATTTTGTAT GAGGCGGTGC AGGGAGACAA ATCACCAATC	TTGGAGCAAA	AATTTTGTAT	GAGGCGGTGC	AGGGAGACAA	ATCACCAATC
01	481	481 CCACAGGCGG TTGATCTGTT TGCTGGGAAA CCACAGCAGG AGGTTGTGTT GGCTGCGCTG	TTGATCTGTT	TGCTGGGAAA	CCACAGCAGG	AGGTTGTGTT	GGCTGCGCTG
	541	541 GAAGATACCT GGGAGACTCT TTCCAAACGC TATGGCAATA ATGTGAGTAA CTGGAAAACA	GGGAGACTCT	TTCCAAACGC	TATGGCAATA	ATGTGAGTAA	CTGGAAACA
	601	601 CCTGCAATGG CCTTAACGTT CCGGGCAAAT AATTTCTTTG GTGTACCGCA GGCCGCAGCG	CCTTAACGTT	CCGGGCAAAT	AATTTCTTTG	GTGTACCGCA	GGCCGCAGCG
	661	661 GAAGAAACGC GTCATCAGGC GGAGTATCAA AACCGTGGAA CAGAAAACGA TATGATTGTT	GTCATCAGGC	GGAGTATCAA	AACCGTGGAA	CAGAAAACGA	TATGATTGTT
	721	721 TICICACCAA CGACAAGCGA TCGTCCTGTG CTTGCCTGGG ATGTGGTCGC ACCCGGTCAG	CGACAAGCGA	TCGTCCTGTG	CTTGCCTGGG	ATGTGGTCGC	ACCCGGTCAG
15	781	781 AGTGGGTTTA TTGCTCCCGA TGGAACAGTT GATAAGCACT ATGAAGATCA GCTGAAAATG	TTGCTCCCGA	TGGAACAGTT	GATAAGCACT	ATGAAGATCA	GCTGAAAATG
	841	TACGAAAATT	TACGAAAATT TIGGCCGTAA GICGCICTGG TTAACGAAGC AGGAIGTGGA GGCGCATAAG	GTCGCTCTGG	TTAACGAAGC	AGGATGTGGA	GGCGCATAAG
	901	901 GAGTCGTCTA GA	GA				

The tight there are the tight that the tight time the tight the tight tight tight tight tight tight.

Table 620: DNA sequence of pCES5 pCES5 6680 bases = pCes4 with stuffers Ngene = 6680 Useful REs (cut MAnoLI fewer than 3 times) Non-cutters	n CDR1-2 and CDR3 2000.12.13	AvrII Cctagg BsmFI Nnnnnnnnnnnnnngtccc BstBI TTcgaa Ecl1361 GAGctc	Kpnl GGIACC Nsil ATGCAt Pmll CACGTG Sacl GAGCTC SexAl Accwggt Spel Actagt Stul AGGcct		2636 4208 148 1156	1667 2347 6137 2321 4245 2321 4245
	sequence of pCES5 bases = pCes4 with) cut MAnoLI fewer tha	c atc	GATatc TGGcca TTAATtaa RGgwccy CCGCgg GCGATCGC	Xmal Cccggg ut more than 5 4 6G 10	ymes that cut from 1 to 3 t 1091 RGgnccy 3 I Ctcgtg 1 Cacgag 1 II Tcatga 3 II GACGTC 1	GTATCCNNNNNN 2 I CTGAAG 1 cttcag 2 Cycgrg 3 AI GWGCWC 3 GWGCWC 1 GWGCWC 1 AGTact 1

1.7

	Xbal Tctaga	-	3767		
	!AflII Cttaag	.	3811		
5	BsmI NGcattc	-	3821		
	i-"- GAATGCN		4695		
	!RsrII CGgwccg	-	3827		
	!WheI Gctagc	-	4166		
	BstEII Ggtnacc	-	4182		
01	Bambi CGTCTCNnnnn	2	4188	6625	
	!-"- Nnnnnngagacg	1	6673		
	!ApaI GGGCCc		4209		
		m	4209	4492 6319	
	Bsp1201 Gggccc	1	4209		
2		1	4209		
	!BseRI NNnnnnnnnctcctc	ctc 1	4226		
	!-"- GAGGAGNNNNNNNNN	NNN 1	4957		
	!EcoNI CCTNNnnnagg	-	4278		
	!PflFI GACNnngtc	1	4308		
20	!TthlllI GACNnngtc	1	4308		
	!KasI Ggcgcc	2	4327	5967	
	BetxI CCANNNNhtgg	1	4415		
	!NotI GCggccgc	1	4507		
	!Eagl Cggccg	1	4508		
25	BamHI Ggatcc	1	5169		
	BspDI Arcgat	-	5476		
	!NdeI CAtatg	-	5672		
	!EcoRI Gaattc	_	5806		
	Psil TTAtaa	1	6118		
30	!DraIII CACNNNgtg	1	6243		
	BsaAl YACgtr	1	6246		
		ן ט	cgcctattt	 t tataggttaa	tgtcatgata ataatggttt
		BssSI. (1/2	<u>.</u>		
35	61 cttaGACGTC	C aggtggcact tttcggggaa	tttcgggga	a atgtgcgcgg	aaccctatt tgtttattt
	! AatII				
	121 tctaaataca !	ttcaaatat	G TATCCgctc BciVI(1 o	ca tgagacaata of 2)	accctgataa atgcttcaat
ç	aat	aa			
40	! Base # 201 to 1061	II	ApR gene from pUC119 with	19 with some	RE sites removed
	1 2	3 4 5	6 7 8	9 10 11	12 13 14 15
	υ γ				

gcg	30 R K aaa	45 atc 60 P	75 C tgt	90 G ggT	105 P cca	120 L tta	135 L tta	150 L ttg	165 P
ttt	29 V gtg	44 Y Y tac 59 R	74 I cta	89 L ctc	104 S Tca	119 E gaa	134 N aac	149 F ttt	164 E
ttt	28 L ctg	43 G ggt 58 F	73 L ctg	88 O CAa :gI	103 Y TAC I	118 R aga	133 A gcc	148 A gct	163 W
ညည	27 T acg	42 V gtg 57 S	72 V gtt	87 E gaG Bc	102 E gAG	117 V gta	132 A gcg	147 T acc	162 R
att	26 E gaa	41 R Cga 56	343 71 K aaa	86 O Caa	101 V gtt	116 T aca	131 T act	146 L cta	161 D
ctt	25 P cca	40 A 9 gcc 55 L	70 F	85 G ggg	100 L ttg	115 M atg	130 N aac	145 E gag	160 L
gcc	24 H cac	39 G G ggt I I	69 T act	84 A gcc	99 D gac	114 G ggc	129 D gat	144 K aag	159 R
gtc	23 A gct	1 L L L L L L L L L L L L L L L L L L L	68 S agc	83 D gac	98 N aat	113 D gat	128 S agt	143 P ccg	158 T
cgt	22 F ttt	37 0 cag 52 6	67 M atg	82 I att	97 Q cag	112 T acg	127 M atg	142 G gga //2)	157 V
ttc	21 V gtt	36 D gat 51	66 M atg	81 R cgt	96 s tct	1111 L ctt	126 T acc	141 G Gga	156 H
cat	20 P cct	35 gaa gaa 50	65 P cca	80 tcc	95 Y tat	1110 H cat	125 I ata	140 I ATC	155 D
Caa	19 L ctt	34 gct 49 L	64 F	79 L tta	94 H Cac	109 K aag	124 A gcc	139 T acc Pvu	154 G
att	18 C tgc	33 D gat 48 D	63 R cgt	78 V gta	93 I ata	108 E gaa	123 A gct	138 T aca	153 M
agt	17 F ttt	32 K aaa aaa 47 L	62 E gaa	77 A gcg	92 R cgc	107 T aca	122 S agt	137 L ctg	152 N
atg	16 A gca	31 V gta 46 E	61 E gaa	76 G ggc	91 R CGC	106 V gtc	121 C tgc	136 L ctt	151 H
201	246	291	381	426	471 .BcgI	516	561	909	
-	رم	07	15	20	25	30	35	40	

double that the mile of comparison for the first of mile of the other sense.

وسياء عليه ابتناء سيح دار مسجد وسيد واستاء القديد القديد المسيد عرب يوسد، وسيد المسيد القديد المسيد المسيد

	,							
	1141	tcctttttga	taatctcatg	accaaaatcc	cttaacgtga	gttttcgttc	cactgagcgt	
	1201	cagaccccgt	agaaaagatc	aaaggatctt	cttgagatcc	ttttttctg	cgcgtaatct	
	1261	gctgcttgca	aacaaaaaa	ccaccgctac	cagcggtggt	ttgtttgccg	gatcaagagc	
	1321	taccaactct	tttccgaag	gtaactggct	tcagcagagc	gcagatacca	aatactgtcc	
	1381	ttctagtgta	gccgtagtta	ggccaccact	tcaaqaactc	tatagcacca	cctacatacc	
	1441	tegetetget	aatcctgtta	ccagtggctg	ctgccaqtqq	cdataaqtcd	tatettacca	
	1501	gqttggacto	aagacgatag	ttaccqqata	addcdcadcd	atcaaactaa	acadadaatt	
	1561	cgtgcataca		qaqcqaacqa	cctacaccda	actdadatac	ctacacctc	
-	1621	agcattgaga	aagcgccacg	cttcccgaag	ggagaaaggc	ggacagGTAT	CCggtaagc	
	,					BciVI	(2 of 2)	
	1681	gcagggtcgg	aacaggagag	cgCACGAGgg aç BssSI.(2/2)	agcttccagg	gggaaacgcc tggtatcttt	tggtatcttt	
	1741	atagtcctgt	cgggtttcgc	cacctctgac	ttgagcgtcg	atttttgtga	tgctcgtcag	
	1801	ggggcggag	cctatggaaa	aacgccagca		tttacggttc	ctggcctttt	
-	1861	gctggccttt	tgctcACATG	Ttctttcctg	cgttatcccc	tgattctgtg	gataaccgta	
•	1921	ttaccgcctt	tgagtgagct	gataccgctc	gccgcagccg	aacdaccdad	cqcaqcqaqt	
	1981	cagtgagcga	ggaagcgGAA	ggaagcgGAA GAGCgcccaa	tacgcaaacc	gcctctcccc	gcgcgttggc	
	2041	cgattcatta	Sapl atgCAGCTGg o	SapI atgCAGCTGg cacgacaggt	ttcccgactg	gaaagcgggc	aqtqaqcqca	
				./3)			1	
-	2101	acgcaatTAA	TGTgagttag ctcactcatt	ctcactcatt	aggcaccca	ggcTTTACAC	tttatgcttc	
	2161	+ 4 + 2 + 4 + 4 + 4 + 4 + 4 + 4 + 4 + 4	35	Plac	4	10. ************************************	C E A E C C A C A A	
_	4 > 4	282522552		ه د دی دی می دی	yaraacaarr	LCACACAGGA A	AACAGCIAIG	
	2221	ACcatgatta	cgCCAAGCTT PflMI	TGGagccttt	TGGagccttt tttttggaga	ttttcaac	10m11d hor-	
	signal:	signal::linker::CLight	Hind3.					
		1 2 3	4 5 6	- K	9 10 11 B I V	12 13 14 V n E	15	
	2269	n gaaaa	tta tta t	gca att	t tta gtt	υ L	tı	
			Linker	er	•	•	End of FR4	
		16 17 18	3 19 20	21 22	23 24 25	26 27 28	29 30	
	2314	S H S tct cac aGT	A GCA	V Q gtc caa	Q V 5 CAG GTC	D L E GAC CTC GAG	I K atc aaa	
		Aŗ	ApaLI		PstI:	XhoI		
					SSPMI SalI AccI	(1/2)		
					HincI	HincII. (1/2)		

		:			
45 S tct	60 L ctg 75 D gat	9 90 Q cag 4 105 L L G CTG	120 V gtc	135 R agg	
44 P	59 L ctg 74 V gtg	89 E E gag 104 T acG	119 E gaa	134 N aac	
43 P	58 C tgc 73 K	88 aca 103 L ctg	118 C tgc	133 F ttc	
 42 F ttc	57 V gtg 72 W tgg	87 V gtc 102 I acc	117 A gcc 2/2)	132 S agc	
41 I atc 1/2)	56 V gtt 71 Q Cag	86 S agt 101 S agc	116 Y TAC (131 K aag	
40 F TTC	55 S tct 70 V gta	85 gag 100 s agc	115 V GTC '	130 T aca /2)	
39 V GTC BbsI	54 960 69 8 K	84 C a g 99 C t C	114 K aaa	129 V GTg	att
38 S tct	53 T act 68 A gcc	83 tcc 98 s	1113 H cac	7 128 P.A. CCG. AgeI	GCCa
37 P cca	52 G gga 67 E gag	82 N aac 97 Y tac	112 K aaa	127 S tcA Ag	GG CGCGCCaatt
36 A gca	51 S tct 66 R aga	81 G ggt 96 T	111 E gag	126 S agt	9
35 A gct	50 K aaa aaa 65 P CCC	80 S tcg 95 S s	110 Y tac	125 L ctg	140 taa
34 V gtg	49 L ttg 64 Y tat	79 C caa 94 D Gac	109 D gac	124 G ggc	139 taa
33 T	48 0 cag 63 F	78 L ctc 93 K R	108 A gca	123 Q cag	138 C tgt
Ckappa- 31 32 R G gt gga	47 E gag 62 N aac	77 A gcc 92 S agc	107 K aaa	122 H cat	137 E gag
Cka 31 R cgt	46 D gat 61 N	76 N aac 91 D gac	106 S AGC	121 T acc	136 G gga
2359	2404	2494 2539	2584 EspI	2629	2674
^ §	5 2	20	30	35	40

:

ctatttcaag gagacagtca ta	reib::3-23(stuffed)::Chl::111 fusion gene 1 2 3 4 5 6 7 8 9 M K Y L L P T A A 2723 atg aaa tac,cta ttg cct acg gca gcc	16 17 18 19 20 21 22 A A Q P A M A gcG GCC cag ccG GCC atg gcc Sfil	FR1(DP4) 23 24 E V gaa gtt	31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 6 G L V Q P G G S L R L S C A ggc ggt ct tta cgt ct ttgc gct	FR1 46 47 48 A S G gct TCC GGA BspEI	Stuffer for CDR1, FR2, and CDR2 There are no stop codons in this	tttgtggggt ggtgcagatc gcgttacgga cttaactgcT GATCAggcat gggatgttat	gaggettttt ttacctacte tgcaageage tegtcagttg gtagaaacat taacaegttg
	9 10 11 12 13 14 15 N A G L L L L L c gct gga ttg tta tta ctc		FR1(DP47/V3-23)	. 40 41 42 43 44 45 1 L R L S C A thtalcgtlcttltctltgcigctl		stuffer. gcttcAGATC Tgtttgcctt	gatcgaccga ctgct tcgccaaacc agtcg	gaggettttt ttacctacte tgeaageage gacatetggt ttgacaeaga gegateegeg tegteagttg gtagaaaeat taacaegttg ggatggeate aatttgetta atgatgatgg

The Unit of the B B B Comp. III. The Book of the Comp. Comp. Comp. Book of the Book of the Comp. Comp. Comp. Book of the Book

ט ט ט	, o b	ις e	0 11	io D			
9 150 S c tcc 4 165		195 S tca	210 S : agc	225 S s agc		150 A gca	165 tag >
149 S tcc 164 V		194 S tcc	209 S agc	224 P ccc	:	149 1 A gcc g	164 1 A gca t
148 P CCC 163 L		193 Q cag	208 5 tcc	223 K aag	238 C TGT		1
147 A gca 162 C	177 N aac	192 L cta	207 P ccc	222 H cac	237 S TCT	7 148 G c ggg	2 163 A g gcc
146 L ctg 161 G	176 W tgg	191 V gtc	206 V gtg	221 N aat	236 K AAA	6 147 H t cac	1 162 G t ggg
145 P CCC 160 L Ctg		190 A gct	205 T acc	220 V gtg	235 P CCC	5 146 H C Cat) 161 N y aat
144 F ttc 159 A gcc		189 P ccg	204 V gtg	219 ; N aac	4- ი.;	nker 145 H : cac	160 L ctg
143 V gtc 158 A gcg	173 T acg e	188 : F	203 ; V gta q	218 2 C tgc a	233 2 V GTT (s lil 144 H Cat	159 D r gat
142 S tcg tcg 157	172 ; V gtg a	187 ; T acc t	202 2 S agc c	217 2 I atc t		Poly His linker 142 143 144 145 H H H H cat cat cac	158 E 1 gag
141 P cca 156 G ggc e		186 : H cac a	201 2 S agc a	216 2 Y tac a	231 232 K K aaG AAA ON-TQF		157 E 1 gaa
140 G G ggc 6		185 J V gtc 0	200 2 L ctc a	215 2 T acc t	230 2 D gac a) 141 A : GCa	s s tca
139 K aag 154 S tct	169 P	184] G ggc c	199 2 S tcc c	214 2 Q cag a	229 2 V gtg g	39 140 A A CG GCC otI	1 155 I c atc
138 T acc 153	168 F ttc c	183 S S agc c	198] Y tac t	213 ; T acc c	228 2 K aag c	139 A GCG NotI Eag	154 I ctc
137 S tcc a 152 S	167 : Y tac 1	182 j T acc a	197	212 2 G ggc a	227 2 T acc a		153 K 1 aaa 1 III
136 A gcc 1 151 K aag 6	166 J D gac t	181 L ctg a	196 1 G gga c	211 2 L ttg c	226 2 N aac a		151 152 E Q gaa caa Mature 1
4198	4288	4333	4378	4423	4468	4507	1: 1 4543 gc
ر. 	01	15	20	25	98	35	40

t Fi t t	195 N aac	210 T act	225 L ctt	240 S tct 255	Y tac	270 D gac	285 N aat	300 Q cag	315 T acg	330 Q
t s t a	194 A gct	209 C tgt	224 G ggg	239 G ggt 254	E gag	269 L ctc	284 P cct	299 F ttt	314 Y tat	329 Y
N aat	193 Y tac	208 ; V gtt	223 I att		Cct	268 P cct	283 N aat	298 M atg	313 V gtt	328 Y
ਜ਼ ਯੁਕਕ ਫ਼	192 : R cgt 1	207 ; V gtg	222 P cct	237 G ggt		267 N aac	282 A gct	297 F ttc	312 T act	327 T
T aca	191 : D gat (206 ; V gtt o	221 ; V gtt (236 E gag		266 I atc	281 P ccc	296 T act	311 L tta	326 K
H Cat	190 J L tta g	205 2 G ggc (220 ; W tgg		act	265 Y tat	280 N aac	295 N aat	310 A gca	325 V
P cct (189 ; T act t	204 ; T aca e	219 ; T aca	234 G ggc		264 T act	279 Q Caa	294 L ctt	309 G ggt	324 P
ж ааа с	188 K aaa	203 ; A GCt ;	218 ; G ggt	233 G ggt		263 Y tat	278 E gag	293 P cct	308 Q cag	323 D
A gca	187 D gac	202 N AAT (217 Y tac	232 G ggt		262 G ggc	277 T act	292 Q cag	307 R agg	322 T
L tta	186 D gac	201 W tgG	216 C tgt		E gag	261 P ccg	276 G ggt	291 S tct (2/2)	306 N aat	321 G
c tgt	185 K aaa	200 L ctg	215 Q cag	230 N aat	s tct	260 I att	275 P cct	289 290 E E 5AG GAG BseRI(305 R cga	320 Q
s agt	184 W tgg	199 C tgt	214 T act	229 E E Gaa	G ggt	259 P cct	274 P ccg	289 E GAG BseF	304 F	319 T
G Gaa	183 V gtc	198 G ggc	213 E gaa	228 P cct	9 9 9	258 T aca	273 Y tat	288 L ctt	303 R agg	318 V
V gtt	182 N aac	197 E gag	212 D gac	227 I atc	ggt ggt	257 D gat	272 T act	287 S tct	302 N aat	317 T
r act	181 T act	196 Y tat	211 G ggt	226 A gct	E gag	256 G ggt	271 G ggc	286 P cct	301 N aat	316 G
4588	4633	4678	4723	4768	4813	4858	4903	4948	4993	
- · •	رم .	02	: :: :: :: :	20		25	30	35	40	

cag	345 N aac	9 360 D G GAT BamHI	375 Q caa 2)	390 G ggc	405 G ggc	420 D gat	435 T acc	450 K aaa	465 F ttc	480 G
tac	344 W tgg	359 E	374 P Cct	389 G ggt	404 G ggt	419 G ggt	434 M atg	449 G ggc	464 G ggt	479 T
tat	343 Y tac	358 N aat	373 L CTG	388 S tct	403 E gag	418 S tcc	433 A gct	448 K aaa	463 D GAT	478 A
act	342 A gct	357 F ttt	372 37; D L gAC CT(BspMI	387 G ggt	402 S tct	417 G ggt	432 G ggg	447 A gct	462 46; I D ATC GA: BSPDI.	477 G
aaa	341 D gac	356 G ggc	371 S tct	386 G ggt	401 G ggt	416 S tcc	431 K aag	446 D gac	461 A gct	476 N
gtt	340 Y tat	355 s tct	370 S tcg	385 G ggt	400 G ggc	415 G ggc	430 N aat	445 S tct	460 A gct	475 G
. ນ	339 M atg	354 H cat	369 Q caa	384 s tct	399 G ggt	414 G ggc	429 A gct	444 Q Cag	459 G ggt	474 N
gac	338 A gcc	353 F ttc	368 G ggc	383 G ggc	398 E gag	413 G ggt	428 N aac	443 L cta	458 Y tac	473 A
act	337 K aaa	352 A gct	367 Q caa	382 G ggc	397 S tct	412 S tcc	427 A gca	442 A gcg	457 D gat	472 L
ggc	336 S tca	351 C tgc	366 Y tat	381 G ggc	396 G ggc	411 G ggt	426 M atg	441 N aac	456 T act	471 G
caa	335 S tca	350 D gac	365 E gaa	380 A gct	395 G ggc	410 G ggc	425 K aaa	440 E gaa	455 A gct	470 S
act	334 V gta	.349 R aga	3.64 C tgt	379 N aat	394 G ggt	409 G ggt	424 E gaa	439 D gat	454 V gtc	469 V
gtt	333 P cct	348 F	363 V gtt	378 V gtc	393 E gag	408 E gag	423 Y tat	438 A gcc	453 S tct	468 D
act	332 T act	347 K aaa	362 F ttc	377 P cct	392 s tct	407 s tct	422 D gat	437 N aat	452 D gat	467 G
ggc	331 Y tac	346 G ggt	361 P CCa	376 P cct	391 G ggc	406 G ggc	421 F ttt	436 E gaa	451 L ctt	466 I
5038	: : 5083	5128	5173 BamHI	: ! 5218	: ! 5263	5308	: : 5353	: : 5398	5443	
	5	01		92		52	30	35	40	

gac gtt tcc ggc ctt gct aat ggt aat ggt gct act ggt	483 484 485 486 487 488 489 490 491 492 493 494 495 A G S N S Q M A Q V G D G gct ggc tct aat tcc caa atg gct caa gtc ggt gac ggt	498 499 500 501 502 503 504 505 506 507 508 509 510 S P L M N F R Q Y L P S tca cct tta atg aat aat ttc cgt caa tat tta cct tct 513 514 515 515 517 518 519 520 521 522 523 524 525	514 515 516 517 518 519 520 521 522 523 524 S V E C R P Y V F G A tcg gtt gaa tgt cgc cct tat gtc ttt ggc gct	528 529 530 531 532 533 534 535 536 537 538 539 540 Y E F S I D C D K I N L F TAT Gaa ttt tct att gat tgt gac aaa ata aac tta ttc	543 544 545 546 547 548 549 550 551 552 553 554 555 V F A F L L Y V A T F M Y gtc ttt gcg ttt ctt tta tat gtt gcc acc ttt atg tat	558 559 560 561 562 563 564 565 566 567 568 569 570 S T F A N I L R N K E S . tcg acg ttt gct aac ata ctg cgt aat aag gag tct taa	c egt cgttttacaa cgtcgtgact gggaaaaccc tggcgttacc caacttaatc agc acatccccct ttcgccagct ggcgtaatag cgaagaggcc cgcacCGATC	cca acagtTGCGC Agcctgaatg gcgaatGGCG CCtgatgcgg tattttctcc FSpI (2/2) tct gtgcggtatt tcacaccgca tataaattgt aaacgttaat attttgttaa gtt aaatttttgt taaatcagct cattttttaa ccaataggcc gaaatcggca TTA TAAatcaaaa gaatagcccg agatagggtt gagtgttgtt ccagtttgga	FSII tcc actattaaag aacgtggact ccaacgtcaa agggcgaaaa accgtctatc tgg ccCACtacGT Gaaccatcac ccaaatcaag ttttttgggg tcgaggtgcc
att ggt	481 482 4 D F gat ttt	496 497 b D N gat aat 1	P Cct	526 527 52 K P Y aaa cCA TA NdeI.	541 542 9 R G cgt ggt	556 557 U V F gta ttt 1	571 taa GAATTC ECORI. actggccgt	Gccttccca (3/3)PvuI (3/3) 5991 ttacgcatct 6051 aattcgcgtt 6111 aaatcccTTT	rsi. acaagagtcc aqqqcqatqq

The first of the state is the first of the state of the s

rgaaaGC NgoMIV	agggcgctgg	gcgccgctac	tctgatgccg	cgggcttgtc	atgtgtcaga	
agcttga cggg	CGGCgaacgt ggcgagaaag gaagggaaga aagcgaaagg agcgggcgct agggcgctgg 1IV.(2/2)	caagtgtagc ggtcacgctg cgcgtaacca ccacacccgc cgcgcttaat gcgccgctac	agggcgcgta ctatggttgc tttgacgggt gcagtctcag tacaatctgc tctgatgccg	catagttaag ccagccccga cacccgccaa cacccgctga cgcgccctga cgggcttgtc	igctcccggc atccgcttac agacaagctg tgaccgtctc cgggagctgc atgtgtcaga	
cccgatt taga	aagcgaaagg	ccacacccgc	gcagtctcag	cacccgctga	tgaccgtctc	
aaggga gcc	gaagggaaga	cgcgtaacca	tttgacgggt	cacccgccaa	agacaagctg	aaacgcgcga
cggaac ccta	ggcgagaaag	ggtcacgctg	ctatggttgc	ccagccccga	atccgcttac	ggttttcacc gtcatcaccg aaacgcgcga
6291 gtaaagcact aaatcggaac cctaaaggga gcccccgatt tagagcttga cggggaaaGC Ng	CGGCgaacgt.NgoMIV.(2/2)	caagtgtagc	agggcgcgta	catagttaag	tgctcccggc	ggttttcacc
6291	6351	6411	6471	6531	6591	6651

and the true and are the first to the sound of the state of the state

630: Oligonucleotides used to clone CDR1/2 diversity equences are 5' to 3'.

N_CD1Bsp, 30 bases

C T C A C T g g C T T C C g g A 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

C A C T T T C T C T 21 22 23 24 25 26 27 28 29 30

N Br12, 42 bases

A A A C C C A C T C C A A A C C 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

T A c c A g g A g c T T g g c g 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36

c c c A 39 40 41 42

N CD2Xba, 51 bases

A A g g c A g T g A T c T A g A 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

T A g T g A A g c g A c c T T T 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36

c g g A g T c A g c A T A 39 40 41 42 43 44 45 46 47 48 49 50 51

N_BotXba, 23 bases

A 18

> g A T A g 19 20 21 22 23

10 End Tables